

Will recession become slump?

Two centuries of self-conscious science and technology may have given the world previously unimagined prosperity; can the same agents of change help avoid the calamity that seems to threaten?

IN spaceflight circles, there is an old joke that when a rocket takes off successfully, the television people describe it as a scientific triumph, but that, otherwise, they call it an engineering failure. A similar partition of responsibility attends the technical community's opinion of the uses to which innovations are put: the discovery of antibiotics, say, is rated an outstanding technical achievement, but the continuing high death-rate from bacterial infections, notably in developing countries, is counted a failure of administrators and politicians. But can the technical community so easily shrug off responsibility?

The present cycle of innovation, beginning with the steam engines and novel farming techniques of the late eighteenth century, differs from previous cycles in two respects: it has been extraordinarily successful, witness at least the rich countries of the world, and self-conscious in the sense that people have been able to guess which innovations would be worthwhile — and have been guessed correctly often enough to justify the present generous support of research and development by governments of all persuasions.

The technical community cannot take all the credit. Had not the banking system already been invented, eighteenth-century farmers would not have been able to afford to let fields lie fallow one year in three, and few of James Watt's customers would have been able to afford a steam-engine. It is no accident that, at about the same time, Adam Smith made explicit how *The Wealth of Nations* rests on the division of labour (perhaps magnified by machines) between specialists. Since then, a third social innovation has been found essential for the sustenance of beneficial innovation: higher education as a means of training people and of gathering new knowledge. All this is so elementary that it is usually forgotten.

That is a great misfortune. Especially now. This year, 1990, widely flagged as that in which the challenge would be to distribute the benefits of the European *rapprochement*, is turning into a nightmare. It is not impossible that there will be both a slump, 1930s style, and a major war. The portents are familiar to all those who read the newspapers. What follows is a brief selection of them from the Northern Hemisphere catalogued by longitude from East to West (with the Sun) beginning at Greenwich:

Europe. The summit meeting of the European Communities (EC) was more than usually a disaster, not only for

the manner of Mrs Margaret Thatcher's familiar expression of continuing disaffection from monetary union, but because the EC failed conspicuously to do what it should have done (settle an agricultural deal to get the Geneva trade talks moving) and instead did what it should not have done (fixed 1994 as the date for the second phase of monetary union without deciding how it will be accomplished). Thatcher's objections, if not their manner, were correct. Meanwhile, Britain is moving smartly into recession and may yet have to make things worse by raising interest rates to meet its new European currency obligations.

Germany, on the other hand, is having to borrow to bankroll its four new eastern provinces, so that interest rates have gone up (worsening others' prospects).

North America. The six-month negotiation between the US Congress and the administration has produced a budget that will leave next year's estimates of the federal deficit roughly unchanged from last month's \$500,000 million, or roughly \$2,000 per head per year. So the process will have to begin again in a few months, and with a Congress that can only have been made more obdurate by the outcome of this week's elections. But now state treasurers have joined the borrowing queue, at a time when the major commercial banks are being forced further to cut back on lending by the wasting of their assets, most recently loans against office blocks and other commercial buildings that nobody wants to occupy. So it is no surprise that the US economy is also heading downwards. Canada, as if eager to deny that nothing ever happens there, is about to embark on another bruising constitutional haggles occasioned by now almost-monoglot French Quebec.

Japan. Industrial production marvellously continues to increase, but wealth-creation probably lags far behind the rate at which assets are disappearing from the balance sheets of financial institutions and the commercial banks. The value of shares traded on the Tokyo stock exchange has already fallen by 40 per cent from its peak last year. The worry now is that the price of land, down by 10 per cent so far, will also begin to slide. Then, as in the United States, Japanese banks would have to cut back further on credit; positive feedback is the key phrase. It would be easier to be sanguine about the future if the government were not openly divided on economic policy — and if it were strong enough either to win parliamentary approval



for sending 1,000 unarmed technicians to the Persian Gulf or to say that it will not bother.

Asia. The ideology that held the Soviet Union together for 70 years has, for the time being at least, become irrelevant, the continued existence of the Soviet Communist Party notwithstanding. But fissiparous tendencies in the republics (now Georgia and Moldavia are on the way to being balkanized, while the Ukraine has issued a kind of supplementary national currency of its own) and the manifest collapse of the system for distributing food and other goods represent obstacles to the patchwork of piecemeal economic reforms Mr Mikhail Gorbachev seems to have substituted for the two comprehensive economic plans between which the Supreme Soviet could not decide last month. In normal times, the decision that foreigners can now own enterprises in the Soviet Union could have been electrifying, but people elsewhere are not now in a mood for new ventures. It remains a puzzle that Gorbachev is grappling with the problems of denationalizing the huge and inefficient state industries when it would be simpler, quicker and more effective to let shops, taxi services and the like make their own way in the free world.

Meanwhile, as if to ensure that it will not be overlooked even in a catalogue of misfortune, India, proudly the world's largest secular democracy, is haunted by sectarian violence costing more than 100 lives a week (still less than 0.1 per cent of the annual increase of population).

Central Europe and the Gulf. From Riga to Riyadh, there is turmoil. Poland, Czechoslovakia and Hungary are learning that freedom is painfully expensive. Prices have also increased in Romania and Bulgaria, so far without much compensating freedom.

The danger is that disappointment will engender regression to the old ways or, worse, to chaos. The good news (last weekend) is that the Warsaw Pact has cut a deal among its members on the redistribution of military equipment; that should improve the chances that a treaty on conventional forces in Europe will be signed at Paris on 19 November. Further south, although the chances that there will be a war diminish as the weeks go by, so do the prospects of a constructive settlement.

There remain three strictly global problems — the threat of global warming, the plan to revise the General Agreement on Tariffs and Trade (GATT) and the condition of the poorest countries.

This week's climate conference (see page 98) vividly illustrates the division of opinion between governments on what should be done and when (if ever). This journal's line is simple: let there be a treaty binding everybody to act when the need is pressing, but do not enter numbers until the important signatures have been collected. If there were a real slump, of course, carbon dioxide emissions would be abated for a time. As things are, many at Geneva say there are even more pressing matters.

The trade negotiation is one. GATT's rules require member states either to agree by the end of the year or to abandon four years of negotiations. The prize is huge —

the extension of free trade (which means the international division of labour) to a new range of goods and, for the first time, services (such as insurance) as well as an international understanding on intellectual property. The EC's failure even to agree how far to reduce its agricultural subsidies (\$97,000 million a year, nearly half from taxes, the rest by way of high prices paid by European consumers) is the immediate stumbling-block. But the United States, which has rightly been pushing for reform, may yet find that the new Congress stymies whatever deal is struck. Either way, failure will make a new wave of chauvinistic protectionism coincide with the worldwide credit squeeze. That, ominously, is what happened in the United States in 1931. If there is a war as well, the wasting of assets will be even more dramatic.

In an ideal world, the problems of the poor countries would be thought even more pressing, yet they will end up on everybody's back burner. Sadly, if they were just money problems, they might have been solved by the unrequited loans the world's commercial banks are busily stripping from their balance sheets, further aggravating the world-wide money squeeze. The conventional wisdom is that they will have to wait.

Sadly, economists' sleight of hand has nothing to offer. Interfering with the natural credit squeeze would merely regenerate the stagflation of the early 1980s. The key issue is the US deficit, hitherto compatible with low inflation by being financed from overseas. If (as seems likely) that no longer happens, and if the deficit continues (recession can only make it worse), either Americans will be impoverished by inflation or the credit squeeze on US enterprises will be intensified. The process should stop when assets are so devalued that they seem cheap again, but there is a long way to go before that happens.

What, in this gloom, can the technical community do? The simple answer ("Nothing") is a cop-out. Does the technical community, while having changed the world, have no influence outside its narrow expertise? Manifestly, that is false; over thirty years, it has powerfully shaped the arms control agreements that would ordinarily have made 1990 memorable. Are jingoism (as at Rome), chauvinism (in GATT) and plain nationalism (in the Gulf and everywhere) necessarily less accessible? It will no longer suffice to say that they are strictly political matters; they are also impediments to the necessary continued division of labour.

Talk by itself will not prevent a slump, of course, but it might, by raising the level of debate, help people see what may be on the way.

What should that be? The effect of a true slump, as in the 1930s, is like that of shuffling a pack of cards: those that end up on top of the pile are usually different from what they were before. So may there not be justice in such a process? Only rough justice, at best. And the 1930s showed that the process of slump is economically painful and socially corrosive. Rational readjustment must surely be preferable. Why, when so much else is rational, should it be unattainable? □

Bush plans expansion of science and computing

- \$1,000 million for high-performance computing
- Pre-college science gets top education priority

Washington

BUOYED by the success of this year's \$1,000 million US global warming initiative, President George Bush is planning to introduce similar initiatives in high-performance computing and science education in his January budget request for 1992, said D. Allan Bromley, the President's science adviser, in an interview last week. In the case of the computing initiative, the President is expected to request more than \$150 million above the \$500 million the federal government is already spending on high-performance computing, a category that includes supercomputers, high-speed net-works and advanced software development.

Funding will rise to over \$1,000 million by 1997, according to a report* issued last year by the White House Office of Science and Technology Policy (OSTP). Bromley said the new initiative will largely follow the recommendations of the report.

Extra funds are also expected to accompany the launch of the science-education initiative. Although the size of the increase is still under negotiation, administration officials say that total funding will be well over the \$1,800 million the federal government now spends.

Both initiatives will combine existing efforts of participating federal agencies, as well as create new programmes, Bromley said. Like the massive global warming initiative, which was the first to mould the research programmes at many agencies to fit one set of specific priorities, the computing and education initiatives will have their goals and agendas set by the Federal Coordinating Council on Science, Education and Technology (FCCSET), a body led by Bromley and made up of top officials from all the science-related federal agencies.

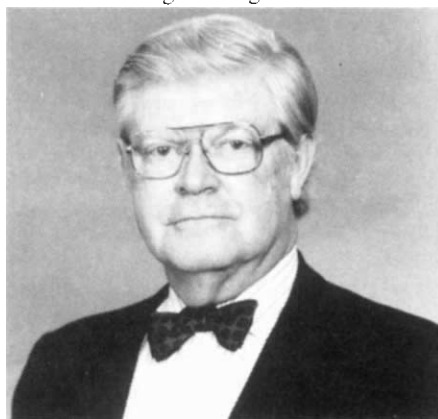
A FCCSET initiative is best for "areas that don't fit comfortably into one or a few agencies, but across many agencies, as well as those that don't fit comfortably into a single discipline", Bromley said. "There is also some presumption that such initiatives should receive increased funding." This year, Congress granted most of the administration's request for a 57 per cent (\$375 million) increase for global warming research over last year.

Encouraged by such success, Bromley said he now considers FCCSET initiatives a "major policy instrument". Future in-

itiatives may be announced in material science and technology and in biotechnology, he said.

High-performance computing

The high-performance computing initiative has been in the making for several years, says Charles Brownstein, National Science Foundation (NSF) acting assistant director for computer and information science and engineering. It will combine



D. Allan Bromley, White House science adviser.

the NSF's four supercomputer centres, the Defence advanced Research Projects Agency's Strategic Computing initiative the "hypercube" computing initiative at the National Aeronautics and Space Administration's (NASA) laboratories and the Department of Energy's (DoE) climate-change computing and magnetic-fusion computing programmes (see *Nature* 344, 277; 22 March 1990). A total of eight agencies will participate, Brownstein says.

Top priorities, according to Brownstein, will include:

- Designing new computer hardware and operating systems, emphasizing "scalable" parallel-processing technology that can be applied to a wide range of high-performance computers. By the end of the five-year programme, researchers hope to have computers whose performance is measured in the "tera-op" range; that is, trillions of operations per second, or 1,000 times more powerful than today's computers.

- Putting these machines in centres such as the DoE's National Laboratories, the NSF supercomputer centres and NASA research centres. All these machines will also be connected to national networks such as the Internet and NSFNet.

- Expanding existing networks and on-line databases. The current NSFNet 'backbone' will soon be at T3 speed — 45

megabits per second. But expansions that will be part of the new initiative will "vastly expand connectivity to literally hundreds of higher-education and pre-college education centres as well as industry", Brownstein says.

- Ten per cent of the initiative's budget will go to "basic research and human resources" — projects aimed at individual investigators, to encourage scientists to take advantage of the high-performance computing.

Education and human resources

The education initiative also reflects the priorities of Congress, although it is solely an administration effort. Because Congress, which has historically ranked education higher than has the White House, tends to appropriate more for education than the President requests, "there's been a proliferation of band-aid programmes"; small projects that may be worthy, but do not necessarily contribute to national priorities, says David Sanchez, NSF assistant director for education: "There are a lot of little programmes that need looking at", he says.

Even with the new initiative, which will be spread across 11 agencies, the federal government's spending will represent less than 10 per cent of the total US education budget, notes Luther Williams, assistant director for education at NSF. At present, federal spending is only 6 per cent of the total, meaning that its role is "catalytic", says Williams. That makes it all the more important that all US science education efforts point the same way, he says.

The top priority for the new initiative will be on pre-college education, which accounts for two-thirds of the NSF budget.

Christopher Anderson

BSE

First non-UK case

Geneva

THE first European case of the cattle disease bovine spongiform encephalopathy (BSE) outside the United Kingdom and the Irish republic has been recorded in a six-year-old cow from the Jura mountains in northwest Switzerland. Two more suspected Swiss cases are being examined.

Peter Gasner, director of the Swiss veterinary office, says the infected cow was Swiss-born, but it is not known whether it had consumed animal feed imported from the United Kingdom. Cattle feed containing offal from infected animals was banned in the United Kingdom after being identified as the most likely mode of BSE transmission. The Swiss case will renew speculation that the disease has occurred undiagnosed elsewhere; Gasner finds it "unbelievable" that BSE is absent from the rest of the European continent.

Peter Aldhous

* The Federal High Performance Computing Program. Office of Science and Technology Policy, September 8, 1989.

Two declarations at odds

Geneva

A PLEA that nations should promptly begin to reduce emissions of greenhouse gases, and strengthen the sinks that remove them from the atmosphere is the centrepiece of the statement adopted by more than 700 scientists at the end of their six-day meeting here on 3 November.

The technical meeting, organized by UN bodies including the World Meteorological Office (WMO), and the UN Environmental Programme (UNEP), was called to consider the reports of the Intergovernmental Panel on Climate Change (IPCC) and to review 10 years of the World Climate Programme. It will have been followed this week by a meeting of government ministers and officials, who will also produce a statement.

The scientists' statement is significantly stronger than assessments of climate change made by IPCC. It says that there are "technically feasible and cost-effective

opportunities" for reducing carbon dioxide emissions in all countries, and that many industrialized nations should be able to reduce them by 20 per cent by 2005.

This view is not likely to be embraced by the ministerial part of the conference. As officials struggled with the drafting of the second statement, several hundreds of metres away from the main conference at the Palais des Nations, the opposition of the United States to targets for carbon dioxide emissions seemed to prevail over the positions of European states already committed to stabilizing emissions.

The need for a statement, ostensibly to pass onto ministers, overshadowed the scientific meeting. The meeting, according to IPCC chairman Bert Bolin was a "funny creature" with little new science.

The final session, at which the details of the statement were hammered out, uncovered the hidden agendas of some of the participants. This, for example, was when Yuri Izrael, chairman of the IPCC working group on the impact of climate change and a prominent member of the Soviet ministerial delegation, tried (with others) to remove the statement on the prompt reduction of carbon dioxide emissions.

The final version is a victory for those allied more or less closely with the environmentalist cause, led by Irving Mintzer, from the University of Maryland, and

Peter Gleick from the California-based Pacific Institute.

In the event, the scientists' declaration will have little bearing on the expected ministerial statement. "You never teach a minister anything at a conference", says Bolin. That statement is expected merely to welcome the commitment by the European Communities and others to stabilize carbon dioxide emissions by 2000.

The final draft presented to ministers on Tuesday urges other developed countries to adopt strategies which will have "significant effects on limiting emissions" other than CFCs and other greenhouse gases not controlled by the Montreal Protocol, with no mention of a timetable.

Simon Roberts of Friends of the Earth, one of many environmental groups represented as observers at the meeting, said that officials negotiating the ministerial statement were "working in a scientific vacuum" by ignoring the scientists' conclusion.

But Howard Ferguson, coordinator of the conference, said that the scientists' declaration should be seen as a "longer-term document" that will influence the governing bodies of environmental research programmes in the coming year. Among other things, the statement urges more climate research and monitoring centred on a new Global Climate Observing System — a comprehensive network of land, ocean and space-based monitoring stations.

Peter Aldhous

The history man

Geneva

MIKHAIL Budyko, from the Soviet State Hydrological Institute at Leningrad, believes that accurate regional forecasts of climate change are already possible by the reconstruction of past climate at times of differing concentrations of carbon dioxide. But Budyko won few converts to his view.

Lacking access to computer power, Budyko and many of his compatriots have concentrated on climate history. His argument last week was based on climate reconstructions for three periods of warming in the Northern Hemisphere: the mid-Holocene (5,000 to 6,000 years ago), the Eemian interglacial (125,000 years ago) and the Pliocene optimum (3.3 to 3.4 million years ago).

Budyko argues that the relative temperature increases at different latitudes are remarkably consistent during the three warming periods, and also claims concordance with low-confidence IPCC regional predictions of warming.

But Budyko's palaeoclimate reconstructions predict much greater increases of precipitation with warming than IPCC's models, especially in mid-latitudes. That leads him to conclude that the productivity of natural ecosystems and agriculture will be enhanced, and that the world economy might benefit from a return to a climatic "Paradise lost". But his current ideas are shared by few climatologists.

Much of the Northern Hemisphere warming and increased rainfall in the Holocene and Eemian was due to changes in the Earth's orbit, rather than carbon dioxide, climate modellers say. P.A.

Climatologists wanted

Geneva

ACCURATE regional forecasts of climate change could be delayed for 20 years by shortages of computing power and trained climatologists, according to leading climate modellers this week. Yet governments may demand regional predictions before committing themselves to stringent action against global warming.

Jerry Mahlman, of Princeton University warned the conference of a tendency to underestimate the real problems modellers face in making regional predictions. The need is for a doubling of the resolution of the climate models on which IPCC's assessment is based.

Running such models will take ten times the computing power now devoted to climate modelling and may not be achieved for ten years, says John Mitchell of the UK Meteorological Office. That bottleneck may be removed with growing international concern over global warming.

But Mitchell also says that the greatest single barrier to accurate regional prediction is the lack of understanding of the physical processes within clouds — turbulence, radiative fluxes and the behaviour of water droplets.

Mitchell's colleague Geoff Jenkins says that the British government could "throw £5 million into these studies" and achieve very little unless there were more researchers. The people needed, physical scientists with a solid training in environmental science, are in very short supply he says. Of some 20 recent recruits at the Meteorological Office's Hadley Centre for climate change research, which opened earlier this year, only three had previous experience in climatology. Mitchell reckons that it takes three years for good young physical scientists to become productive in the field.

The problem seems to be global. Gordon McBean, chairman of the steering committee of the World Climate Research Programme, fears that "we may end up with more funds than people to carry out the work". He wants to see the bridging of the gap between the physical and environmental sciences in most countries' educational systems. But he sees a ray of hope in US plans to begin new postgraduate and postdoctoral programmes geared to the interpretation of the vast quantities of data expected from remote-sensing satellites.

Peter Aldhous

Pleas made for science spy

Paris

THE French League of Human Rights has decided to take up the case of Rolf Dobbertin, a plasma physicist at the Centre National de la Recherche Scientifique (CNRS), who was sentenced in June this year for spying for East Germany. Last month, the case provoked an open letter condemning the magistrate's decision from 18 members of the Académie des Sciences and the Collège de France, including Nobel laureates Jean-Marie Lehn and François Jacob. Although Dobbertin admits having had relations with East German intelligence, the documents he sent were widely available, unclassified preprints of scientific articles.

Dobbertin was born in the former German Democratic Republic and began working in a CNRS laboratory at the University of Paris VII in 1962. In 1979 he was arrested by French counterintelligence under Article 80 (III) of the Penal Code, for acts of "intelligence with agents of a foreign power that are harmful to the military or diplomatic situation of France or to its essential economic interests". Remanded in custody for four years, Dobbertin appeared before a now-defunct military court in 1981. After other court appearances, he was finally released on bail by an appeal court in 1983 pending his trial. In June this year he was finally tried and sentenced to 12 years imprisonment.

At a recent press conference held by French League of Human Rights, it emerged that scientists are less upset by the plight of Dobbertin — although the sentence was felt to be "excessive" — than by the apparent assault the court ruling makes on the freedom to disseminate scientific information. At Dobbertin's trial, Professor René Pellat, president of the CNRS, confirmed that the documents recorded on the microfilm were "explicitly destined for the general scientific public" and that passing them on was "like sending a newspaper". At the appeal court hearing, in 1983, the prosecution lawyer admitted the documents were public, but said "the confidential or secret nature of the supplied documents is of no interest in characterizing the crime".

According to J. Micheli, of the Committee for the Liberation of R. Dobbertin, the trial "was indirectly a trial of the free circulation of scientific information". And speaking at the offices of French League of Human Rights last week, Professor Y. Queré, a physicist at the Académie des Sciences, said "the tradition of exchange between scientists dates back to the Renaissance. It is one of the basic conditions for the development of science".

It seems that it was Dobbertin's use of microfilm and the fact that his correspon-

dents were secret agents rather than scientists that upset the French authorities. Although scientists calling for Dobbertin's release do not defend his unusual actions, they claim he has committed no crime. Professor E. Schatzman, of the Academy of Sciences, said that Dobbertin is a theoretical physicist and that, although there could be potential applications from his research field, "there is a wide gap between a formula and its application". Yves Jouffa, president of French League of Human Rights, says "he is not accused of spying but of having contact with intelligence of a foreign power. How can he be condemned to 12 years in prison without having passed any secrets?"

In a letter addressed to the French newspaper *Libération* and sent from prison, Dobbertin defends his use of spy

technology: "the dissemination of science using documentary film, practised by libraries the world over" is not a medium "distinctive of espionage", he says.

Apart from the "general principle" of scientific freedom, the academicians' letter protests against Dobbertin's sentence. "Coming over 15 years after the deeds concerning a state which has practically ceased to exist, while, over the past seven years, the accused has been able freely to continue his research activity at CNRS, the extremely heavy 12-year prison sentence makes this trial belong to another age".

A petition for Dobbertin's release has received over 800 signatures and scientists have been sending preprints to Dobbertin in prison.

A delegation from the French League of Human Rights will meet President François Mitterrand shortly to ask for Dobbertin's release and pardon.

Peter Coles

UNIVERSITIES FUNDING COUNCIL

Sparks fly in funding muddle

London

THE disarray in funding arrangements for universities in the United Kingdom was compounded by strong language last Friday after an emergency meeting of the Committee of Vice-Chancellors and Principals (CVCP). In an open letter to Lord Chilver, Chairman of the Universities Funding Council (UFC), CVCP Chairman Sir Edward Parkes criticized the UFC's earlier rejection of the efforts of universities to comply with the UFC's own new arrangements for apportioning funds as "a serious blow to the effective management of the universities."

Under the UFC's new 'bidding' system (see *Nature* 348, 3; 1 November 1990), universities told the UFC how many students they wished to teach in each subject up to mid-1995, and the price at which students could be taught. The UFC said it would then allocate funds over the four-year period according to its assessment of these bids.

Universities duly tendered their bids, but on 23 October, UFC Secretary Finlay Scott wrote to university vice-chancellors expressing "disappointment" that 93 per cent of bids for student places for 1994–95 were pitched at the 'guide prices' set by the UFC, and effectively suspending further discussion on university funding until after the 1991–92 grant is set in February. The bids assume that student numbers will grow by 19 per cent by 1995.

The CVCP's emergency meeting was held on 2 November to consider this new move. In view of the fact that the UFC declares that it must be satisfied that the government's desire for expansion does not compromise academic standards,

Parkes writes that "it would have been irresponsible for institutions to bid below the guide price in all but a few cases".

In the meantime, universities may take matters into their own hands rather than wait until the UFC allocates next years' grants. University College (UCL), a constituent college of the University of London, is thinking of raising a levy on students in Medicine and Law to augment fees paid on students' behalf by the government.

Dr Stephen Montgomery, Director of External Relations at UCL, stresses that the college is still discussing the concept as one of several ideas to raise money. "No decisions have been made at this time", he says. Like many other colleges, UCL's financial future is "in a state of uncertainty" following the UFC's 23 October letter.

In the latest issue of UCL's monthly newsletter, UCL's Provost Dr Derek Roberts blames the government for creating a climate in which universities are expected to pick up the bill for the government's aim to increase student numbers. In this way, he says, the government "wishes to be seen as a concerned facilitator whilst we attract the flak". He reserves his greatest ire for the "misinformed" government Public Accounts Committee and its criticism of university management.

"How any bunch of politicians has the nerve to accuse another group of incompetence in the light of successive governments' mismanagement of the UK economy for the last century would be hilarious if it were not so serious", he writes.

Henry Gee

RIKEN sees the light?

Tokyo

WITH the opening last month of a new centre for photodynamics research near Sendai in northern Japan, the Institute of Physical and Chemical Research (RIKEN) has again proved itself to be an odd fish among Japan's many government-funded research laboratories. RIKEN's choice for director of the new institute is Junichi Nishizawa, one of the most outspoken critics of the Japanese research system in general and of the Ministry of Education, Science and Culture in particular.

The new institute is one of several initiatives with which RIKEN has tried to break away from the decaying facilities and ageing populations of researchers that plague government institutes and the national universities. Apart from the new centre in Sendai, RIKEN established a centre for life science research in Tsukuba, north of Tokyo, in 1984, and is now in the process of building the world's most powerful synchrotron, SPring-8 (Super Photon Ring-8 GeV), at Harima Science Park City west of Osaka, in collaboration with the Japan Atomic Energy Research Institute.

In 1986 RIKEN introduced a novel programme, the Frontier Research Program, which employs teams of young researchers under short-term contracts to study the brain, new materials, and homeostasis (*Nature* 352, 569; 1987). Several of the teams are led by foreign scientists, something that is normally unheard of in Japan.

The new Photodynamic Research Centre in Sendai is part of the Frontier Research Program and will contain four laboratories: the laboratory for sub-millimetre waves, which will aim at developing new technology, in particular communication technology, for use in the range 100 GHz to 1 THz; the laboratory for photophysics, which will focus on non-linear interactions between light and inorganic materials, such as ionic crystals, with the ultimate aim of developing optical-computing devices; the laboratory of organometallic photodynamics for design of novel high-performance photofunctional materials; and the laboratory for photobiology, which will examine the physiological functions of light in living organisms.

The centre is expected to have an annual budget of close to ¥600 million (\$4.5 million) from the Science and Technology Agency (STA) from next fiscal year. The local prefectural and municipal governments in the Sendai area are providing land and a new building for the centre free of charge. Construction of the building is expected to begin shortly. Industry will also contribute "some significant support" for research at the centre, according to Minoru Oda, director of RIKEN.

Oda attributes RIKEN's unusual adaptability to the institute's past history. It was established as a private foundation and, before the Second World War, received most of its income from patents on its own inventions. After the war RIKEN had to be radically re-organized because of anti-cartel laws introduced by

JAPANESE UNIVERSITIES

New money and new ideas

Tokyo

JUNICHI Nishizawa last week found himself both director of RIKEN's Photodynamics Research Centre and president of Tohoku University in Sendai — a remarkable pair of posts for a man who has spent his research career collecting foreign prizes for his optoelectronics research and criticizing the uncreative atmosphere of Japanese universities and research institutes, an atmosphere which drove him to set up his own independent institute.

Among Nishizawa's prizes is the prestigious Jack A. Morton prize from the US Institute of Electrical and Electronic Engineers, which he received for the invention of the static induction transistor.

In a recent interview in the Japanese monthly magazine *Voice* he lambasted Japanese university researchers for "heading in the wrong direction" by rushing to work on discoveries made in the West "just as a new gold discovery attracts thousands of prospectors who all start to dig in the same place". In Nishizawa's view, success in basic research can be achieved only by "patiently digging an area no one has ever dug before".

Now that he has joined the establishment, he still intends to find his way around the conservative Ministry of Education, Science and Culture (MESC) by using the new RIKEN institute to help bring non-MESC money to the university. Nishizawa says that MESC was initially opposed to his idea of bringing the RIKEN photodynamics centre to Sendai. But he points out that he is simply returning RIKEN to its roots (RIKEN was provisionally established at Tohoku Imperial University before moving to its present site near Tokyo).

And he says that he has repeatedly told MESC that they should never have allowed the Science and Technology Agency (STA) to take over RIKEN after the war and that it was only because of "idleness" on their part that STA succeeded in gaining control of the institute.

the US occupation forces (the US army also dumped RIKEN's cyclotrons in the sea on the grounds that they might be used to make nuclear weapons). Now RIKEN gets about 90 per cent of its funds from STA. Nevertheless, RIKEN still has comparative freedom to establish new laboratories and research programmes when compared with other government-funded research laboratories. Oda says STA gives RIKEN this freedom because "they have learned this is the best way".

David Swinbanks

Three out of five professors at the new centre will be from Tohoku University, and the other two will come from the Wako campus of RIKEN. The centre will have a total complement of about 30 researchers, Nishizawa says.

In addition to the new centre, Nishizawa hopes to bring other STA money to the university, for example through STA's ERATO programme (Nishizawa himself has held two ERATO awards). And he is also making arrangements for industry to fund new chairs at the university. Tokyo University already has several such positions (*Nature* 348, 7; 1990).

But unlike the chairs at Tokyo, which are primarily short-term contract positions for foreigners, Nishizawa intends to create long-term posts for Japanese professors.

Nishizawa says he is also strongly proposing to MESC that the ministry should double present funds for competitive research grants to about ¥100,000 million a year (a proposal that has also been put forward by other Japanese academics — see *Nature* 339, 575; 1989). He says that many people agree with him, including powerful people in the Ministry of Finance and the Diet, but MESC has yet to accept his proposal.

With the new money Nishizawa says he hopes to promote research that relies on "modest serendipity", like that which led to the discovery of high-temperature superconductors at IBM Zurich, rather than ploughing money into buying expensive new equipment as Japanese professors are apt to do. He says the United Kingdom is a model of such research and he would like Japan to learn from the UK system.

But to promote creative research of the type envisioned by Nishizawa requires not only money but a complete overhaul of the organization of the university research system, in order to release young researchers from the control of professors.

David Swinbanks

Bills pass at last minute

Washington

BEFORE rushing home last week for election campaigns, congressional legislators passed some key science bills in a flurry of last-minute voting, but left others to expire when the 101st Congress formally concludes in January.

Top of the list of successes was the passage of the Clean Air Act, after a decade of hard-fought lobbying by environmentalists. The new law will phase out ozone-destroying chlorofluorocarbons (CFCs) over the next decade, eliminating them completely by 2000. CFC substitutes, which are less harmful but still deplete ozone and contribute to the greenhouse effect, will be phased out by 2030. New automobile emission standards will cut hydrocarbon emissions by 35 per cent and nitrogen oxide output by 60 per cent. Smoke-stack emissions will be regulated to reduce sulphur dioxide and nitrogen oxide and decrease acid rain.

Many of the elements of the measure will have global, as well as local, impact, says Richard Ayres, an attorney for the Natural Resources Defence Council. Improved automobile efficiency reduces total US carbon dioxide emissions, and encourages other countries to follow suit with high-mileage cars aimed at the US market. "There's a real international effect from these national standards", he says.

On the research front, the surprise loser was legislation that would have made it a federal crime to break into an animal-research laboratory (see *Nature* 344, 96; 1990). Although one bill had already been passed by the senate and another by Representative Charles Stenholm (Democrat, Texas) appeared on the verge of passage in the house, "we just ran out of time", says Barbara Rich, a National Association for Biomedical Research lobbyist. Similar bills will be reintroduced early next year, but animal activists are promising a stiffer fight, and prospects of quick passage are slim.

For biotechnology, modification to the orphan drug act was the only significant legislation to emerge. The 1983 Act gave companies financial perks and exclusive marketing rights for drugs that would probably be otherwise unprofitable, due to their small potential markets (see *Nature* 345, 759; 1990). But critics complained that it was virtually a licence to print money for some fortunate companies, whose drugs became unexpectedly profitable after they had already been designated "orphans."

Early in the year, Henry Waxman (Democrat, California) introduced a bill that would modify the act by making orphan status rescindable when drugs become money-makers on their own and

allowing some competition between companies who were developing drugs concurrently. Despite objections from some companies that he was changing the rules in the middle of the game, Waxman compromised only enough to include a clause for companies with orphan drugs already on the market or in the pipeline. The bill, which leaves untouched the blockbuster drugs that sparked off the controversy, will now become law.

Two legislative proposals, a fetal tissue research bill (see *Nature* 346, 598; 1990) and the Human Genome Privacy Act (see *Nature* 347, 221; 1990), were introduced at

the eleventh hour. A proposal to end the indefinite moratorium on federally funded fetal tissue research failed to be passed — due to Congress's inability or unwillingness to deal with the issue of fetal tissue transplantation research. It will be taken up again in January 1991.

The Human Genome Privacy Act, a bill proposed by Representative John Conyers (Democrat, Michigan) to prevent government disclosure of genetic information to third parties without the individual's consent, may fare better. It is backed by a broad coalition of scientists, ethicists and religious leaders, and will be reintroduced next year, his staff says.

Christopher Anderson & Diane Gershon

US SCIENCE FUNDING

Harnessing northern lights

Washington

IMAGINE this: A powerful US senator puts \$9 million dollars in next year's budget for your university's research and gives you a \$25 million supercomputer. But then he



The aurora borealis (or northern lights).

specifies that you use the money and the machine for an impossible project: to tap the energy of the aurora borealis to provide clean power and fight global warming. "Harness the electrojet — this inexhaustible supply energy in space — and bring it to earth", he asks.

This nightmare of pork-barrel politics became a reality for the University of Alaska's Geophysical Institute last month when Senator Ted Stevens' (Republican, Alaska) auroral-research proposal slipped past Congress in the final stages of the budget process. Scientifically, admits Geophysical Institute director Syun Akasofu with some understatement, extracting energy from the northern lights is "a dream". Still, \$34 million is a hard gift to

refuse. After some thought, Akasofu and his staff came up with an idea that would enable them to accept the money with clean consciences. Noting that the aurora is created when charged particles are captured by the Earth's magnetic field, Akasofu issued a statement last week saying that, "What the Senator means by 'bringing aurora energy down to the ground' is to 'bring the knowledge of auroral research to accelerate fusion energy research'".

Knowledge of magnetically steered plasmas is needed both to understand the aurora and to build a fusion machine, Akasofu points out. Thanks to \$10 million received with Stevens' help last year, his institute is already at work on a high-powered radio transmitter that may be able to modulate the aurora so that it acts as a huge low-frequency antenna. (The plan is to use the antenna so that the Navy can communicate with its submarines while they are underwater.) Modulating one kind of plasma is not so different from controlling another, Akasofu says, adding that his centre has collaborated with fusion scientists from the Princeton Plasma Physics Laboratory and Los Alamos National Laboratory. "We're trying to understand the basic nature of hydrogen plasma — surely that will help the fusion people", he says.

But Akasofu may still have some trouble getting his money. In the closing hours of the budget appropriations process last week, Senator Sam Nunn (Democrat, Georgia), the powerful head of the defence committee, attacked pork-barrel projects in the defence budget, and promised to hold hearings to review them early next year. "I intend to hear directly from the Secretary of Defence, to determine his views on each and every one of the earmarked projects", he warned.

Christopher Anderson

Russians to the rescue

San Francisco

SOVIET scientists have announced their intention to contribute \$200 million worth of support to one of three US consortia hoping to win contracts for the construction of the Superconducting Super Collider (SSC)'s giant particle detectors. This contribution to the SSC is much the largest to be registered so far from outside the United States.

The Soviet offer was announced at the end of last month by Michael Marx, professor of physics at the State University of New York at Stony Brook, who is coordinating the EMPACT/TEXAS consortium. (EMPACT stands for Electrons, Muons and Partons Using Air Core Toroids.) Marx reported that a draft agreement to provide up to \$200 million worth of "in-kind" support to his group's project was signed by Nikolai Tyurin, vice director of the Soviet Union's Institute for High Energy Physics (IHEP) in Protvino, 60 miles from Moscow. The EMPACT project includes 200 US scientists, as well as Brazilian and Mexican collaborators.

Budget and planning decisions about the SSC itself are still up in the air, but plans for two large particle detectors are moving ahead, because their construction may take nearly as long as construction of the SSC itself. Planners hope to have the 54-mile accelerator, to be located outside Dallas, Texas, built and running by 1999 at a cost of about \$8,250 million.

So far, three groups of physicists have expressed interest in building a detector. They are now preparing 'letters of intent', and the SSC's Program Advisory Committee will recommend two of the three for approval, perhaps as early as December. Each approved group expects to receive from general SSC funds about half of the \$500 million cost of building a detector. The remainder is to come from foreign contributions.

Leaders of each of the three groups report that they are working on multinational collaborations, which include Soviet scientists, and that they expect to receive in-kind contributions from the Soviet Union and elsewhere if their proposals are approved. But so far only Marx's group has a firm promise of large-scale Soviet support. Tyurin leads a team of about 120 scientists at 12 Soviet institutions who would take part in the project. Physicists from IHEP, the USSR's largest high-energy physics laboratory, have already collaborated successfully on the D₀ detector project at Fermi National Accelerator Laboratory in Batavia, Illinois, where they have provided both equipment and scientists.

Although the agreement for Soviet contribution to the EMPACT/TEXAS

detector is between scientists, not governments, Marx said his group has discussed the project with Soviet government officials, and fully expects their cooperation if the project is approved. Details of the Soviets' contributions have not yet been established, but the \$200 million figure is based on the expectation that participation will be in proportion to the number of scientists involved in the project.

The competing groups must, in their letters of intent, present a "plausible" plan for the funding of their projects, said Jack Sandweiss, Yale University physicist who chairs the Program Advisory Committee. The Committee will meet in mid-December to evaluate the letters of intent.

The EMPACT/TEXAS detector is a relatively specialized one, particularly suited to searching for the elusive Higgs particle, which Marx expects to be produced at a rate of fewer than 100 per year.

Other projects competing for approval are the Solenoidal Detector Collaboration (SDC) led by George Trilling of the Lawrence Berkeley Laboratory of the University of California, and the Lone Star Detector headed by Samuel Ting of the Massachusetts Institute of Technology.

Over 500 physicists are participating in the SDC project, according to Trilling, including about 300 Americans, 90 Japanese, about 60 Soviets as well as Canadians and Europeans. The SDC is the only project considered a "general purpose" detector, and would have very broad abilities to track all the particles produced by the SSC's proton collisions. Ting's proposed detector, which would specialize in the detection of muons, electrons and positrons, involves about 1,000 people, including 200 to 300 Americans, 300 Soviets and an array of Europeans and Asians. Ting expects that many of the countries that now collaborate with him on the Large Electron-Positron (LEP) collider outside Geneva will also contribute to his SSC detector if it is approved. He said his group's proposed SSC detector will be "similar in philosophy" to the detector that he directs at LEP.

After two of the detector groups are selected, they will be asked to develop formal proposals over the course of about a year, and the group leaders predict that detector construction will begin between late 1991 and late 1992. Foreign governments are not expected to make any official commitments until the two projects have been approved. Nevertheless, Marx, who admits his group began as a "tremendous underdog" in the competition, says he is very optimistic since his Soviet colleagues have been willing to put pen to paper in support of his group's plan.

Elizabeth Schaefer

Million-dollar quark

Washington

ALL previous records for a publisher's advance on a science book have been broken in an international auction that has already gathered more than one million dollars for rights to a book planned by Nobel laureate Murray Gell-Mann, professor of theoretical physics at the California Institute of Technology.

According to Gell-Mann's agent, John Brockman, who is based in New York, advances for *The Quark and the Jaguar: Adventures in the Simple and the Complex* are "way over a million dollars" for just the US and German rights, sold to Bantam Books and R. Piper respectively. In a series of auctions, Brockman has sold the book to publishers in 11 other countries, including Macdonald in the United Kingdom and Editions Albin Michel in France.

"Five years ago if you had asked me if a book like this would be the hit of the Frankfurt Book Fair, I would have thought you were crazy", says Brockman. But the success of Stephen Hawking's *A Brief History of Time*, changed publishers' perceptions of serious science books. Precise sales figures for Hawking's book are difficult to come by, but his British publisher, Bantam UK, says that "about 250,000 copies" were sold in Britain, and "around a million" in the United States before the book went into paperback this summer.

Brockman points to the success of James Gleick's *Chaos*, Richard Feynman's autobiographical books, Roger Penrose's *The Emperor's New Mind* and Clifford Stoll's *The Cuckoo's Egg* as evidence of a trend towards success for "books with serious content". All of these spent months on the *New York Times* non-fiction best-seller list.

So great is the confidence in the popularity of science that Gell-Mann's book was sold on the basis of a 32-page proposal alone. He intends to complete the manuscript by the spring of 1992 for publication by autumn 1992. The book is described as "a personal voyage of scientific discovery", which will go beyond Hawking's description of the attempt to formulate the fundamental laws of physics and, Gell-Mann says, "try to answer the question: What if we know these laws? What comes next?"

Recovering a multi-million dollar advance may not be as difficult as it seems. Brockman points out that with a 15 per cent royalty (a cut above the 5–10 per cent payable to less royal authors) on a \$20 book, sales of only around 300,000 — far less than those attained by *A Brief History of Time* — are needed before the work goes into paperback. That is, of course, if a new fad has not come along by the publication date in autumn 1992.

Alun Anderson and Tim Lincoln

Battle over censorship

San Francisco

IN an action that may cause a fundamental change in the way government research contracts are written in the United States, Stanford University has filed a lawsuit to prevent federal government control of publication of research results. Stanford alleges that the National Heart, Lung, and Blood Institute (NHLBI), a part of the National Institutes of Health (NIH), violated the University's rights to free speech granted by the First Amendment when it withdrew an offer for a research contract.

Stanford had refused to sign the agreement if it contained the stipulation that the government had the authority to censor publication of the research results. In the suit filed on 24 October, the University is seeking to have the \$1.4 million contract returned to Stanford, and to stop the Department of Health and Human Services (DHHS), which oversees NIH research contracts, from demanding such an agreement in future contracts with any institution. The suit, filed on behalf of Stanford's Board of Trustees, named as defendants DHHS; NHLBI; Louis Sullivan, secretary of DHHS; and Claude Lenfant, director of NHLBI.

The 'Confidentiality of Information Clause', a DHHS regulation which NHLBI insisted be included in the contract, violates Stanford's policy of openness in research, which was adopted in 1969, by stating that research results could not be published if the agency posed any written objections. The university's suit maintains that when NHLBI withdrew the contract offer on 31 August for the sole reason that Stanford objected to the clause, the "defendants punished Stanford for asserting its constitutional right to free speech". Six days after the contract was withdrawn from Stanford, it was awarded to St Louis University Medical Center.

The dispute arose over a contract offered by NHLBI to Stanford for clinical trials in patients of a permanently implantable heart pump known as the Left Ventricular Assist System (LVAS), to be conducted by Philip Oyer, a professor of cardiovascular surgery in the Stanford University School of Medicine. Oyer has worked on development of the device for almost 20 years. Patients awaiting heart transplants have been successfully sustained at Stanford and elsewhere by a temporary version of the LVAS, manufactured by Novacor, a division of Baxter Healthcare Corp. The long-term version is to be tested for patients with end-stage heart failure for whom an appropriate donor cannot be located.

Most American research universities have policies similar to Stanford's rules on

openness in research, according to Robert Rosenzweig, president of the American Association of Universities. Stanford's policy guidelines on secrecy in research require that all interested parties have freedom of access to research data, processes and results. It also states that Stanford will not enter into any research programme that permits an outside sponsor to restrict publication.

According to Stanford's complaint, the DHHS regulation requires the confidentiality of information clause be included in contracts when personal privacy or proprietary information must be guarded, or when public disclosure of preliminary unvalidated findings "have the possibility

SCIENCE IN GERMANY

Clean slate for academy

Munich

IN an act signalling that science in eastern Germany requires a clean break with the past, the West Berlin parliament agreed on 26 October that the learned society of the East German Academy of Sciences should be dissolved if it failed to reform itself speedily and completely. The 70 research institutes that formed the backbone of the academy have already been removed and attached to the new East German *Länder* while their future is being decided.

The society includes not only local and foreign members chosen for their scientific achievement but also institute directors who received their positions through accommodation with the Communist regime. Many have managed to retain their posts as well as their membership in the society.

The decree was introduced into the West Berlin parliament at the urging of enraged members of the East Berlin parliament, some of whom are researchers who suffered at the hands of the Communists. Hilde Schramm, a member of the left-wing *Alternative Liste* party that co-sponsored the bill, says that West Berlin parliamentarians wanted to move on to other business, but the East Berliners insisted that the decree be passed before the parliament adjourned in late October. The West Berlin decree calls upon the city government to disband the learned society of the academy with all of its members but at the same time to "preserve the tradition-rich body" for a new set of members. The society traces its history back to 1700, when it was founded under the guidance of Gottfried von Leibnitz as the 'Brandenburg Society of Sciences', also known as the Prussian Academy. The society will probably be recast as an 'Academy of Sciences in Berlin and

of adverse effects on the public or the Federal agency". After the contract was awarded to St Louis University, Stanford officials filed a protest with the General Accounting Office (GAO). That office dismissed the protest, stating the First Amendment challenge issued by Stanford was a matter for the courts.

At issue in this case is the question of whether First Amendment rights can be contracted away, according to Robert Charrow, former principal deputy counsel general for DHHS. He notes that in cases of national security these rights can be removed by a contract. Thus, while the parties agree that research data must be handled responsibly, Stanford's challenge may determine whether such biomedical research can be classified by a government rule.

Elizabeth Schaefer

Brandenburg'. Nevertheless, maintaining the tradition while expelling all the members will be a difficult goal.

Academy President Horst Klinkmann, with the help of a 20-member committee, is frantically trying to prepare a new statute for the society with revised criteria for membership in time for consideration by the government of a reunified Berlin due to be elected on 2 December. Earlier, Klinkmann had tried without success to persuade all current members of the learned society to accept 'non-active membership' while new members were installed. But the plan, which would probably not have survived the scrutiny of Western academies, failed when a number of hard-liners refused to give up their memberships.

One organization that will have a strong say in the structure of the new society is the 'Conference of Academies of Arts and Science's in the Federal Republic of Germany', based in Mainz, which has five member academies in the western *Länder*. The chairman of the conference, Gotthard Schettler of Heidelberg, says that the Berlin society must conform to the standards of political and scientific autonomy set by the other members if it is to be accepted and funded, as the other academies are, by the *Bund-Länder Konferenz*, a body which is funded equally by the federal government and the *Länder*. Schettler says that the majority of the members of the learned society are "scientifically not qualified" and among the ones who are qualified, many "are politically highly suspect". Therefore, he recommends that the members who are nominated for re-election to the reformed academy submit to an outside review. "If they want to keep the old *Kader* (party members), then we won't play along", says Schettler.

Steven Dickman

Public health in Italy

SIR—The Italian Chamber of Deputies has recently approved and forwarded to the Senate a bill to 'reform' the Servizio Sanitario Nazionale (SSN, the Italian National Health Service). The bill confirms the status of the Istituto Superiore di Sanità (ISS, the Italian National Institute of Health) as a "technical-scientific body of SSN" under the authority of the Minister of Health. It also prescribes that regulations should be rewritten so as to endow ISS with "scientific, organizational, financial and book-keeping autonomy". This is obviously a positive decision. ISS, which is well known internationally, is responsible for basic and applied research in biomedicine and public health as well as a host of advisory and regulatory activities. Many of these tasks require a rigorous code for dealing with possible conflicts of interest, both between different public organizations and between public and private special-interest groups.

The hard truth, however, is that the resources allocated by the state budget to ISS for the performance of its increasing duties have become less and less adequate. The figures speak for themselves. With a permanent (tenured) staff of about 1,450, including about 260 scientists and almost 100 directors of research, the total resources each year are at present about L85,000 million (about £38 million or \$74 million). This allocation is intended to cover the salaries and related expenses of tenured personnel (about two thirds of the total) and all other expenses, except two special projects (AIDS and tumour therapy).

In such a situation, there has been an escalation in the chase for "extra-budget" resources earmarked for research agreed with the contracting parties. Under present laws, these agreements can be made with Italian public institutions (for example, the National Research Council and the regional authorities), with foreign organizations (National Institutes of Health) and with international organizations (World Health Organisation, European Communities). The total additional resources acquired in this way are now about L40,000 million a year. Much of this money supports on a temporary basis more than 500 junior scientists and technicians, better known as "the precarious ones" (*i precari*).

The next step in this fight for survival is the attempt to modify the law so as to allow ISS to strike similar bargains with private groups. After considerable controversy, an *ad hoc* provision has been included in the bill approved by the Chamber of Deputies, to become law if the Senate agrees. In an institution such as ours, conflict-of-interest rules would often

be violated under such an arrangement.

On the other hand, it is widely known (and officially acknowledged) that our health services waste many of their resources each year through sloppy management, satisfying customers, prescription of costly medicines of unproven therapeutic value and over-prescription or misprescription of equally costly medicines. Our Treasury continues year after year to show that it is incapable of recovering losses due to fiscal evasion, while our public debt continues to increase.

At this point, scientists will draw the sad conclusion that public institutions endowed with adequate scientific know-how and with sufficient freedom to speak out on important issues without strings are not wanted on the Italian scene.

On 31 October, the *ad hoc* Senate Committee preliminarily approved an additional clause in the bill, specifying that the SSN should earmark for biomedical and public health research not less than one per cent of its total budget. At the present level of expense, this means not less than L600,000 million per year (about £270 million or \$520 million). If this provision is confirmed, and if objective criteria are adopted for partitioning the funds between ISS and other institutions, we might at last be able to rely on a regular source of financing for research, in addition to other sources covering fixed expenses.

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SIR—I was saddened to read the letter of the four professors from the University of Rome (*Nature* 345, 658; 1990), and the subsequent correspondence. I cannot but agree with the issues put forward in the first letter, and in that published in *Nature* 347, 325; 1990. As a young Italian physician who has gone abroad to obtain clinical experience and the opportunity to carry out less parochial research, I cannot understand Cattani's attitude towards publishing in a language more widely read than Italian, considering that he himself publishes widely in English. Most Italian journals are not listed in the *Index Medicus*, many do not practise peer review and, when an abstract in English is presented, it is seldom translated accurately.

A clinical associate professor has plenty of clinical duties, but even though by law it is not allowed, private medical practice constitutes the bulk of a professor's work load, and provides a handsome income, often several times greater than that earned from the university appointment.

Finally, the letter by Drs Borgia, Daga and Martinuzzi (*Nature* 347, 325; 1990) points out the problems faced by young biomedical researchers working as post-graduate and postdoctoral clinical fellows,

possibly in teaching and senior positions, when trying to return home. We are generally regarded as a threat to the heads of department, who fear that our track record may displace them from the limelight, or endanger the chances of their own relatives obtaining a position, if we are judged fairly. We are a precious asset to be regarded dearly, as we are used to looking at things critically, and have been training hard to increase our ability to perform research while on the typical clinical commitments of, for example, the British medical system, where a week of 100-plus working hours is regarded as normal.

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Heavenly hosts

SIR—In the attempt to save certain species from extinction, for example the California condor, the black-footed ferret and so on, how much attention is being given to their natural parasites?

When all of the last remaining members of a species are taken into captivity, they may lose their parasites, either by the drastic change in living conditions, by treatment from zoo veterinarians or by generations of captive breeding. When they, or their offspring, are then released back to the wild, will they be able to become reinfected? Some parasites are quite host-specific in the wild and may indeed become extinct when their natural hosts are gone.

"So what?" may be a typical reaction. But, if our goal is to conserve biological diversity, then indeed all species should be considered, not just those with the most outward appeal. Many hosts evolved or, better still, co-evolved with their parasitic burden. Perhaps they deserve each other.

Equal rights for parasites!

DONALD A. WINDSOR

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Doing it wrong

SIR—It is becoming increasingly difficult to communicate scientific ideas in a form that allows them to be readily understood. Take, for example, the peer report on a manuscript submitted by us to an international journal. "The manuscript is written in a relaxed, conversational style that leads the reader to feel uncertain concerning the rigor with which the experiments were performed." On the one hand, scientists are being urged to report their work in a way which is more universally accessible. On the other . . .

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The metre and the pendulum

J. H. Freeman

In 1790 the French Assemblée Nationale set in train a process that would result in the metric system. But for quirks of history, the course of metrication might have had an Anglo-French dimension — and a different outcome.

NINETEEN eighty nine and 1990 have been marked by a striking contrast in the manner in which the bicentenaries of two related and singularly French happenings have been commemorated. The first — the Revolution of 1789 — was celebrated with a pageant of festivities which culminated in a Parisian extravaganza of *Son* (Jessye Norman's resounding rendering of La Marseillaise in the Place de la Concorde) et *Lumière* (the Arc de Triomphe ablaze with fireworks). The second — the laying of the foundations for the metric system in 1790 — has attracted scant, if any, attention.

There is historical precedent for this, in that metrication was not seen as a matter of any great moment at the time of its inception. It was only one of many similar proposals for reform, among which were an ill-fated ten-hour clock, and a circle divided into 400 degrees (*grades*), as well as an unpopular Republican calendar that must have caused untold confusion until it was abolished by Napoleon in 1805. The Republic's new calendar year, which began on 22 September (the Autumn equinox), was divided into twelve renamed months (January became Pluviose; April, Floreal; and July, Thermidor), each divided again into three ten-day periods (*decades*) with five (or in leap years six) additional Republican fête days. Thus, some ten years after the Revolution, 8 July 1799 was the 20 Messidor in the year seven.

In France at large, metrication was no more popular than the revolutionary calendar. The system faced considerable public resistance, and there was a serious setback in 1812 when, because of continuing opposition, the government of the day backed down and authorized the use of traditional weights and measures alongside the new system. But it survived and went on to flourish. It has since moved gradually but inexorably to its present quite universal acceptance as the foundation of the SI system of units (*Système Internationale d'Unités*).

In common with other European countries, before 1790 France had a system of weights and measures which had evolved over centuries and which reflected the varying needs of commerce, agriculture and industry. Thus the Parisians had their *pouce*, *pied-de-roi* and *perche* which were similar although not identical to the British inch, foot and perch. Discrepancies such as these were an impediment to inter-

national commerce, but the problem was much more serious within France because each province had its own jealously guarded and largely irreconcilable set of standards. The Parisian foot was eleven per cent longer than that of Strasbourg, but ten per cent shorter than that in Bordeaux, and the differences in the various measures of weight and volume were even greater.

The need for a more rational and unified system had long been recognized and the Revolution provided the opportunity to take action. In 1790 an act of parliament (*"Loi relative aux mesures à prendre pour arriver à la fixation de l'unité naturelle des poids et mesures"*) was passed which provided the statutory framework and which gave to the Académie des Sciences the responsibility of introducing a unified system of weights and measures.

In March of the following year the Assemblée Nationale accepted the Académie's proposal that a universal and invariable quantity — one quarter of the

Earth's meridian — should be used as a basis for the new system of weights and measures. The Académie also recommended that this distance should be established with the greatest possible degree of accuracy by undertaking a precise measurement of the length of the arc of the meridian between Dunkirk and Barcelona. And the academicians showed considerable foresight when they envisaged that the proposed new system would have the advantage of being internationally acceptable.

The measurement, which began in June 1792, was clearly going to be a lengthy undertaking. In consequence, in 1793 (year two of the Republic), the Convention, which by then had replaced the Assemblée Nationale, decided to introduce an interim scheme based on an earlier French survey of the geodesic line between Dunkirk and Collioure in the south of France. The linear unit — one ten millionth part of one quarter of the Earth's meridian — which was derived



« — Dites-moi donc ! m'ame Gavin, en v'là des inventions ! j'vas m'acheter une robe et on me parle étranger ; ils me baragouinent des Mètres, des Thermomètres et des Baromètres !... a-t-on vu ça !... »

— Et moi donc ; la fruitière au lieu de quatre onces de beurre, elle m'emberlificote avec des Grammes ! des Filigrammes et des Programmes ! »

(Loosely translated, the exchange runs: "You'll never guess, Mrs Gavin, what's new! I went to buy myself a dress and they spoke to me in a foreign language. They talked gibberish about metres, thermometers and barometers! . . . Would you believe it! . . . "What about me: the grocer, instead of serving me with four ounces of butter, bamboozled me with grammes, filigrammes [untranslatable] and programmes!")

The enduring pains of going metric. Left, a cartoon from France (1840); below, one from the United States (1986). Both are reproduced from *L'Aventure du Mètre*, published by the Musée National des Techniques - CNAM, Paris, last year.



"We're studying the metric system in school.
I weigh 24 kilometers Celsius."

from this exercise was designated as the metre. Related units of volume (the cubic metre) and mass (the weight of a cubic metre of water) along with their various decimal derivatives were also defined.

But these were turbulent times, and the characterization of the definitive unit of length did not escape unscathed. Although French science and technology largely enjoyed a privileged position during the periods of disorder and instability that followed the Revolution, the attitude of the public was at times ambivalent. The dissolution of the Académie des Sciences in August 1793 was a serious setback and in the following year Lavoisier, who had made a major contribution to the characterization of the kilogramme, was sent to the guillotine with the damning judgement that "*la République n'a pas besoin de chimistes, la République n'a pas besoin de savants*". And because of the tumult in Europe, the trigonometric surveys that had begun simultaneously at Dunkirk and Barcelona had to be abandoned. Nevertheless they were finally completed in 1798 and they allowed the distance between the North Pole and the Equator to be estimated as 5,130,740 *toises* (1 *toise* = 6 *pieds*). This was then used to define the metre which became the standard of length for the whole of France in 1799.

As the exercise drew to a close, it also assumed an international complexion when France invited its European allies and some neutral countries to Paris in 1798 to participate in the determination of the definitive standards. But Britain, at war with France, was of course excluded, and subsequent British adhesion to the metric system has been slow — metrication was given facultative status in 1897 but it was officially adopted only in 1965.

It is therefore ironic that Britain might have had an opportunity to be involved at the beginning of the exercise, and as a full partner, in what could have become the *ne plus ultra* of Anglo-French scientific collaboration. The act of 1790 that designated the Académie des Sciences as its agent went on to decree that the King of France should be asked to write to the King of England to request the cooperation of the British parliament in the venture, with the intention that it be then undertaken under the patronage of the two countries by a joint commission of the Académie in Paris and the Royal Society in London.

Such a proposal was not quite as surprising as it might seem. As the *Philosophical Transactions of the Royal Society* of 1742 show, there was already a history of effect-

PROCLAMATION

D U R O I,

Sur le Décret de l'Assemblée Nationale, du 8 Mai 1790, concernant les Poids & Mesures.

Du 22 Août 1790.

L'ASSEMBLÉE NATIONALE désirant faire jouir à jamais la France entière de l'avantage qui doit résulter de l'uniformité des poids & mesures, & voulant que les rapports des anciennes mesures avec les nouvelles soient clairement dé-

Créte ensuite que le Roi fera également supplié d'écrire à Sa Majesté Britannique, & de la prier d'engager le Parlement d'Angleterre à concourir avec l'Assemblée Nationale, à la fixation de l'unité naturelle de mesures & de poids; qu'en conséquence, sous les auspices des deux Nations, des Commissaires de l'Académie des Sciences de Paris pourront se réunir en nombre égal avec des Membres choisis de la Société Royale de Londres, dans la fin.

Extracts from the act of 1790, with the decree (bottom) that the King should be asked to write to the King of England as the first step in the process of both countries' adopting the metric system.

ive cooperation on comparisons of the weights and measures of the two countries:

An Account of the Proportions of the English and French Measures and Weights, from the Standards of the same, kept at the ROYAL SOCIETY.

Some curious Gentlemen both of the ROYAL SOCIETY of London, and of the ROYAL ACADEMY of SCIENCES at Paris, thinking it might be of good Use, for the better comparing together the Success of Experiments made in England and in France, proposed some time since, that accurate Standards of the Measures and Weights of both Nations, carefully examined, and made to agree with each other, might be laid up and preserved in the Archives both of the ROYAL SOCIETY here, and of the ROYAL ACADEMY of SCIENCES at Paris: Which Proposal having been received with the general Approbation of both those Bodies, they were thereupon pleased to give the necessary Directions for the bringing the same into Effect.

Presumably Louis XVI, who was not deposed until 1792, did take some action but no record of any ensuing correspondence has turned up either in France or in the archives of the Royal Society. It is true that the King was soon to have much more serious preoccupations, and it is also the case that there would be a serious deterioration in relations between Britain and France; but not before a detailed consideration of the new system of weights and measures had been undertaken by the Académie. (As it happened, a degree of Anglo-French scientific relations was

maintained in wartime in a manner that would be inconceivable in later years. For example, in 1807 Sir Humphry Davy was awarded the Prix Napoleon of the Institut de France; and that award was followed in 1813 by a tour of Europe during which he went to Paris to meet prominent French scientists and to be presented to the Empress Marie-Louise.)

It thus remains unclear if a formal offer to collaborate in metrication was ever made and, if it was, what response it evoked in Britain. Sadly, France went ahead alone. Two hundred years later we can do no more than speculate on the possible consequences of such an extraordinary joint venture.

Had Britain become actively involved, the subsequent adoption of an international standard would almost certainly have been swifter because of the widespread use of the British Imperial System of weights and measures in other countries.

And as the Assemblée Nationale act of 1790 also shows, the resulting framework of weights and measures might have been very different from the now familiar SI system. Indeed, if Anglo-French cooperation had proceeded as intended we could now be measuring volumes in cubic pendules and distances in kilopendules.

This is because at the time of the French Revolution there were two well-established measurements of length considered to be suitable as the primary standard. That based on the Earth's meridian was chosen in 1791 on the recommendation of a commission set up by the Académie des Sciences. But the other, the length of a pendulum (*pendule*) with a period of one second — the familiar grandfather clock — had been actively considered in a number of countries since about 1670. It was a convenient concept and one that was easy to grasp. Moreover, as the text of the act of 1790 shows ("*pour déterminer . . . la longueur du pendule, et en déduire un modèle invariable pur toutes les mesures et pour les poids . . .*"), it was the pendulum and not the geodesic measurement that was designated by the Assemblée Nationale for the proposed joint action with the Royal Society. □

J.H. Freeman, who until recently was Counsellor for Science and Technology at the British Embassy in Paris, will shortly take up a British Nuclear Fuels Chair in the Department of Electronic and Electrical Engineering, University of Salford, Salford M5 4WT, UK.

ACKNOWLEDGEMENTS. I thank Mme D. Ferriot and M.A. Pommier of the Musée National des Techniques in Paris, and S.J. Cox of the Royal Society in London, for their help in providing the material on which this article is based.

Should camp-followers be policemen?

Journals are bound to be concerned with and by the published products of scientific misconduct, but there must be doubts about their ability to keep a diverse profession's conscience.

Bethesda

THAT the scientific literature is occasionally contaminated with fraudulent or otherwise misleading documents has regrettably become a fact of life. To what extent are journals and, specifically, their editors, able to prevent that by the elaboration of suitable procedures? And are they willing? These were among the questions raised by the Office of Scientific Integrity (OSI) of the National Institutes of Health (NIH) at a small and private conference of a score of journal editors here last week. What follows is not so much a report of the proceedings (of which nothing is attributable to anybody), but an impression of them.

Journals naturally have a vivid self-interest in the avoidance of being stung; too many *errata*, some of which may amount to retractions of published claims, are bad for a journal's reputation, leading would-be contributors to send their manuscripts elsewhere. But is there a wider responsibility, perhaps to the cause of "scientific integrity" as such? And can it be exercised effectively?

Some principles are generally accepted, although not universally practised. Journals should, for example, offer means by which others than the original authors can comment on published research. Otherwise, misleading claims to priority (arising perhaps from oversight) or errors of fact and interpretation cannot be corrected. But that is a bare minimum.

Many US-based journals seem anxious to go — and seem to have gone — a lot further. One objective is to stamp out "honorary co-authorship", the name given by Feder and Stewart (*Nature* **325**, 207–214; 1987) to the practice in which senior researchers add their own names to research reports which are largely the work of lesser fry. The Darsee case shows how honorary co-authorship can land everybody in the soup.

Now, guidelines developed by what is known as the Vancouver group, latterly the International Committee of Medical Editors, are being widely accepted. Among other things, they require all the authors of a research report to attest that they have "participated sufficiently" to "take public responsibility" for its content. Authors must affirm that they have been engaged in the conception and design of an investigation and/or in the analysis and interpretation of data, that they have helped in drafting the report

and that they have seen the final version.

Only to have helped recruit funds or collect data is not sufficient, nor is "general supervision".

These requirements are sensible, yet tougher than they may seem. Physicians who have collected blood samples for a genetic study, for example, or researchers who have contributed to a successful study only DNA clones or specific monoclonal antibodies, would not qualify as authors. And what would happen to the long lists of authors heading reports of experiments in high-energy physics, some of whom may have been responsible only for the design of some crucial electronic component? No recognition?

The trouble with rules is the seemingly recurrent need to break them. Of course, the names of laboratory chiefs should not be added *ex officio* to a list of authors in a research report, nor should those concerned wish that to happen. (The now-recognized dangers may help warn them off.) And people who contribute only materials to other peoples' studies should be thanked for their generosity, not made into fictitious authors.

But what should happen if a scientist, say an amateur, has gathered a mass of empirical data he is not technically competent to analyse? Should credit lie solely with the academic who finally makes sense of it, as these rules would require?

Why should authorship matter so much? Sadly, there are two distinct answers. On the idealistic view of the scientific process, which is the progressive but tentative deepening of understanding, it may be a practitioner's only lasting reward.

But in the world of immediate rewards — promotions, appointments and research grants — authorship has become an indicator of performance. One of the few jokes at an otherwise over-solemn meeting was the dictum that "every editor should have a pimp as a brother so that he will have somebody to look up to". Only a few at last week's meeting seemed concerned (to continue the image) that the scientific literature is in danger of being prostituted to the preferment business.

On the contrary, most speakers seemed content with the guidelines, even to wish that there were more of them. (In the merciful absence of working researchers, one speaker went so far as to say that "medical editors are the keepers of the conscience...".) But a brief discussion of

whether it might be possible to list authors' names so that the relative importance of their contributions would be apparent by inspection ran into the sands of infeasibility.

The Vancouver group has now added the requirement that it should be a precondition of publication that authors should be prepared to share research materials with others, and has won the adherence of the Society of General Microbiology and its nine powerful journals. (Others appear already to have consulted attorneys on the problems of enforcement.) There is general applause for data-sharing, through data banks and otherwise, tempered by an acknowledgement that it is rarely as simple as it seems.

Why all the fuss? At least in the United States, there is a genuine difficulty: creeping litigiousness. Presumably it is only a matter of time before some journal is sued by somebody inconvenienced by a mistaken claim. But, for the time being, legal action swirls around researchers, at least one of whom has been sentenced to six months in jail for falsifying a grant application to a federal agency by citing his own earlier work, afterwards shown to be fraudulent.

The growing apparatus of investigative committees (not to mention OSI's own existence) is how institutions must demonstrate their own vigilance, and thus their continued eligibility for federal grants, but there are problems of due process (for accused researchers) still to be ironed out. Meanwhile, it appears that journals would be legally well-advised to agree on "industry standards" so as to be less vulnerable to defamation suits when, for example, reporting what the committees have to say.

Litigiousness evokes a normative response. That, at least, is the charitable explanation of US institutions' rule-making tendencies. This journal, while no less eager than the rest to encourage seemly behaviour, will rather rely on exhortation and the occasional admonitory illustrative example. As in the past, it will look into suspicious circumstances arising from its own postbag. But rules run the risk of burying what remains the chief function of the literature, that of assisting the communication of information among astonishingly creative people — and between them and their successors.

John Maddox

A slow but restless ridge

Ken C. Macdonald

ALTHOUGH the Mid-Atlantic Ridge spreads at a rate which is nearly an order of magnitude slower than the fast-spreading East Pacific Rise (15–35 mm yr⁻¹ as opposed to 100–170 mm yr⁻¹), it is by no means inactive or quiescent. Smith and Cann report on page 152 of this issue¹ that the ridge axis is riddled by a multitude of recently active volcanoes which are far more numerous than on the East Pacific Rise. Also in this issue², on page 149, Brozena and White report that the location of the Mid-Atlantic Ridge has changed by rift propagation, a process documented to be common on fast-spreading centres but thought to be less common at slow-spreading centres.

A simple view of sea-floor spreading has upwelling magma and sea-floor volcanism filling the crack between two plates which are separating at rates of tens of millimetres a year. Thus the creation of new sea floor mimics a giant conveyor belt. This simple view is a reasonable first approximation to the creation of oceanic crust along fast-spreading segments of the mid-ocean ridge away from ridge axis discontinuities, where individual segments are typically 10–100 km long (corresponding to the width of the conveyor belt). Active volcanism is dominated by linear fissure eruptions along crests of axial volcanoes tens of kilometres long. The similarity between these highly elongate axial volcanoes and terrestrial shield volcanoes (as found in Hawaii) has fostered an analogy between the two which is not entirely valid, in my opinion.

The axial 'volcano' actually consists of two plate edges which are buoyantly supported by magma and hot rock; it is not a constructional edifice whose 300–600-m elevation is caused entirely by extrusive volcanism and local thickening of the crust. If spreading were to stop and the magma and hot rock beneath the ridge were to cool and freeze, the axial volcano would largely disappear; this would not be the case for a shield volcano.

Creation of oceanic crust on slow-spreading segments occurs at many small equidimensional seamounts, suggesting that there are numerous 'point sources' of volcanism¹. In contrast to the long axial volcanoes of the East Pacific Rise, these small seamounts are entirely constructional, and their height can be accounted for entirely by extrusive volcanism (Fig. 1). The seamounts are so numerous that they appear to coalesce into a three-dimensional patchwork quilt of lozenge-shaped crustal units. Thus one might expect oceanic crust created at slow-spreading centres to be far more heterogeneous than at fast-spreading centres. Indeed, studies of sea-floor magnetization^{3,4} indicate that the magnetic structure of oceanic crust created on the Mid-Atlantic Ridge is far more heterogeneous than that created on the East Pacific Rise (Fig. 2).

Seamounts are also observed on the East Pacific Rise, but they occur away from the axis and tap magma reservoirs that are separate from that of the axial neovolcanic zone^{5,6}; they bear more

resemblance to large off-axis seamounts in the Atlantic than to the many small near-axis seamounts which Smith and Cann¹ have found.

A ridge segment may lengthen by rift propagation⁷. As it rips through old oceanic lithosphere, creating a new spreading centre oriented perpendicular to a new spreading direction, a neighbouring spreading segment stops spreading and dies. Although as early as 1971 Vogt⁸ suggested that some aspects of rift propagation arise in the development of the North Atlantic, it was not thought to be a common process at slow-spreading ridges. Brozena and White² find that this process is common in the slow-spreading South

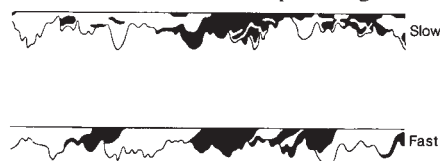


FIG. 2 Statistical studies of sea-floor magnetization; dark areas are normal polarity and light areas are reversed polarity (from ref. 4 and after ref. 3). A wider zone of crustal accretion and more sporadic volcanism may produce greater heterogeneity at slow-spreading rates than at fast as indicated by areas of mixed polarity. Distributed point sources of volcanism¹ at slow-spreading centres helps to explain these differences.

Atlantic along portions of the ridge which are near major hotspots. These hotspots are thought to increase the flux of heat and magma to nearby ridge segments so that they may behave more like fast-spreading centres where rift propagation is known to be common.

On the other hand, in our recent study of the South Atlantic^{9,10}, we found that rift propagation is also common at 25–28° S and 31–34° S where hotspots do not seem to influence the ridge. But rift propagation at these sites tends to be slower and of shorter duration than near the hotspot dominated portions of the ridge. Although there are many important differences in the tectonics of rift propagation at fast- and slow-spreading centres, it is an

1. Smith, D.K. & Cann, J.R. *Nature* **348**, 152–155 (1990).
2. Brozena, J.M. & White, R.S. *Nature* **348**, 149–152 (1990).
3. Schouten, H. & Denham, C.R. in *Deep Drilling Results in the Atlantic Ocean: Ocean Crust*, Maurice Ewing Ser., vol. 2 (eds Talwani, M. et al.) 151–159 (AGU, Washington DC 1979).
4. Macdonald, K.C., Miller, S.P., Luyendyk, B.P., Atwater, T.M. & Shure, L. *J. geophys. Res.* **88**, 3403–3418 (1983).
5. Barone, A. M. & Ryan, W. B. F. *J. geophys. Res.* **95**, 10801–10827 (1990).
6. Fornari, D. J., Perfitt, M. R., Allan, J. F. & Batiza, R. *Nature* **331**, 511–513 (1988).
7. Hey, R.N., Kleinrock, M.C., Miller, S.P., Atwater, T.M. & Searle, R.C. *J. geophys. Res.* **91**, 3369–3393 (1986).
8. Vogt, P.R. *Earth planet. Sci. Lett.* **13**, 153–160 (1971).
9. Carbotte, S.M., Macdonald, K.C. & Welch, S.M. *Mar. geophys. Res.* (in the press).
10. Grindlay, N.R., Fox, P.J. & Macdonald, K.C. *Mar. geophys. Res.* (in the press).
11. Macdonald, K.C. et al. *Nature* **335**, 217–225 (1988).
12. Langmuir, C. *Nature* **326**, 15–16 (1987).
13. Macdonald, K.C. *Nature* **339**, 178–179 (1989).
14. Detrick, R. S., Mutter, J. C., Buhl, P. & Kim, I. *Nature* **347**, 61–64 (1990).

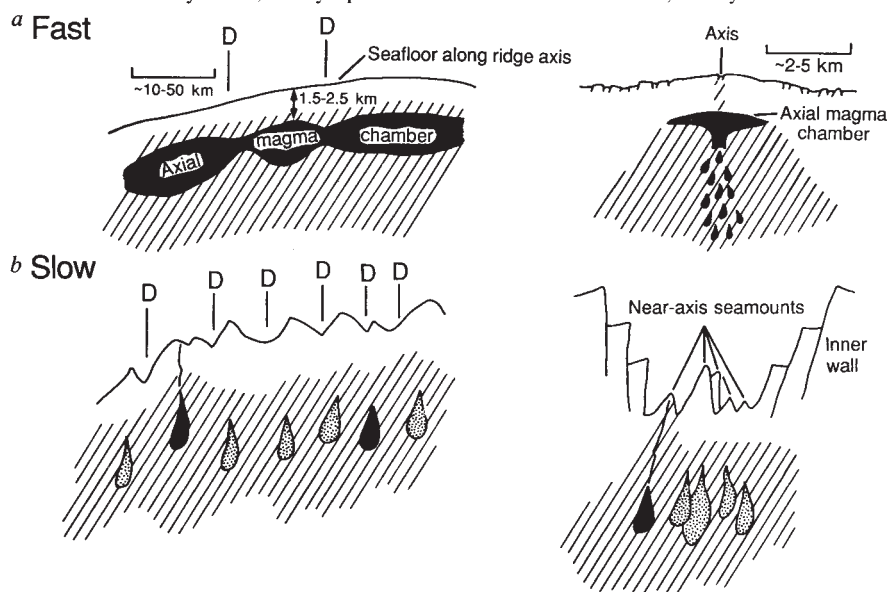


FIG. 1 Possible differences between sources of volcanism for (a) fast and (b) slow-spreading ridges (a, after refs 5, 6, 11–14). Left, schematic cross-sections along-axis; right, perpendicular to the spreading axis. Hatched areas indicate regions of hot rock (near its solidus), dark areas indicate magma; in b, stippled areas indicate recently frozen magma pockets and 'D's' indicate very small (fourth-order¹¹) ridge-axis discontinuities.

important observation that rift propagation can occur at the full range of spreading rates. A curious property of rift propagation which I have noticed in my own work is that the long-term rate of propagation is often approximately 50–100 per cent of the full spreading rate of the ridge, regardless of the spreading rate. The

new South Atlantic studies lend further support to this yet-to-be-explained observation. □

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How efficient can you get?

Mario Capecchi

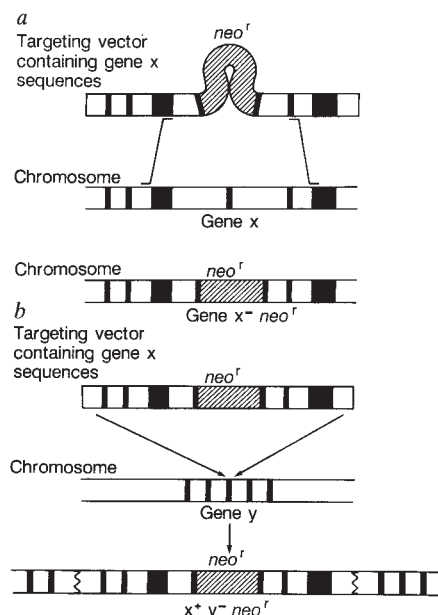
In the tropics, trypanosomatid protozoa cause numerous severe human diseases, including African sleeping sickness, Chagas' disease and kala-azar disease. Many genes have been identified in these protozoa and for some species there are detailed descriptions of the mechanisms of antigen variation and RNA processing. But little is known about the biological function of the cloned genes. This is mainly because of the difficulty of carrying out genetic analysis in these organisms, which have complex biological cycles involving more than one host and which, when propagated in cell culture, do not exhibit a sexual cycle.

A solution to this problem has just been achieved. On pages 171 and 174 of this issue, Cruz and Beverley¹, and ten Asbroek, Ouellete and Borst², report on targeted gene replacement in *Leishmania major* and *Trypanosoma brucei*, respectively. The high efficiency of targeted mutagenesis found by both groups makes it feasible to inactivate both copies of any chosen gene in these diploid organisms. The technology should prove valuable not only for defining gene function but also for combating these organisms in the fight against the diseases they cause. For example, creating truly attenuated strains of *Leishmania* should prove to be useful for the development of vaccines. Even for trypanosomes that exhibit antigen variation, such an approach might prove helpful — in these cases, by concentrating on attenuated strains of metacyclic variants, which are the first to colonize the blood and represent only a subset of the total antigen repertoire, clinically applicable vaccines might be obtained.

After the introduction by electroporation of a targeting vector containing a neomycin resistance gene (*neo*^r) flanked by dihydrofolate reductase thymidylate synthase sequences (*dhfr-ts*), Cruz and Beverley found that 45 to almost 100 per cent of the G418-resistant *Leishmania* colonies contained the predicted homologous replacement of the endogenous *dhfr-ts* sequence with the exogenous sequence. Similarly, ten Asbroek *et al.* targeted the *neo*^r gene into the endogenous tubulin gene cluster. What I find particularly intriguing is the very high

efficiency of these homologous recombination events.

In simple eukaryotes such as yeast³, and now *Leishmania major* and *Trypanosoma brucei*, homologous recombination can predominate over nonhomologous recombination. In organisms of slightly greater complexity, *Dictyostelium* for example, the ratio of homologous to nonhomologous recombination events, following the introduction of exogenous DNA, is roughly 1:5 (ref. 4). In mamma-



a, Homologous recombination (gene targeting). The targeting vector, a neomycin resistance gene (*neo*^r) flanked by gene x sequences is shown pairing with a chromosomal copy of gene x. Homologous recombination between the targeting vector and genomic x DNA results in the specific disruption of the chromosomal copy of gene x (that is, x⁻ but now *neo*^r). b, Nonhomologous recombination (random integration). In nonhomologous recombination the targeting vector is randomly inserted into the chromosomal DNA (here arbitrarily shown to be inserted into gene y). The cells are still x⁺ because the targeting vector was inserted somewhere else in the genome. Most nonhomologous or random insertions of the targeting vector occur through the ends of the targeting vector, presumably at an existing double-strand break in the chromosomal DNA. The solid boxes represent exons, the open boxes introns.

lian cells this ratio is about 1:1,000 (ref. 5). This shift in the ratio does not represent a greater efficiency of homologous recombination in simple eukaryotes but rather an increase in the efficiency of nonhomologous or illegitimate recombination in more complex organisms.

Why have complex organisms developed such an efficient machinery for mediating nonhomologous recombination? Although it is always dangerous in science to approach questions of 'why' rather than 'how', an answer might reside in the relative investment of the species in creation of an individual. Both types of somatic recombination process, homologous and nonhomologous, are part of the repair machinery used by all organisms to counteract the continual damage to DNA resulting from exposure to chemicals and radiation. A double-strand break in the DNA, induced by such agents, results in the loss of all genes distal to the centromere. In single-cell eukaryotes, such a break might result in the death of the cell. But relative to a complex organism, little has been invested in the creation of a single-cell individual.

A similar double-strand break in the DNA of a cell from a complex organism, if it occurs relatively early during embryogenesis, might also result in the loss of the individual. But here the investment is much greater. Under these circumstances, a strategy to save the individual might be to jam the pieces of DNA together by nonhomologous recombination thereby preventing the loss of distal genes. Homologous recombination, in using the information from the other allele, could then be used to repair the damage at the newly formed junction. Compounding the problem faced by more complex organisms is the higher probability of acquiring deleterious double-strand breaks in DNA as a consequence of their having larger genomes.

With these latest successes of gene targeting in *Leishmania* and *Trypanosoma*, there are now even greater incentives to develop the technology in organisms of intermediate complexity such as *Caenorhabditis elegans* and *Drosophila*. The ratio of homologous to nonhomologous recombination in these organisms might prove to be greater than in mammalian cells, thereby helping in the application of gene targeting to certain kinds of genetic analysis. □

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1. Cruz, A. & Beverley, S.M. *Nature* **348**, 171–173 (1990).
2. ten Asbroek, A.L.M.A., Ouellete, M. & Borst, P. *Nature* **348**, 174–175 (1990).
3. Hinnen, A., Hicks, J.B. & Fink, G.R. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1929–1933 (1978).
4. De Lozanne, A. & Spudis, J.A. *Science* **236**, 1086–1091 (1987).
5. Thomas, K.R. & Capecchi, M.R. *Cell* **52**, 503–512 (1987).

Complementary endeavours

Kevin Davies

IN just a little over a year since the gene responsible for cystic fibrosis (CF) was finally identified¹⁻³, substantial progress has been made in several key areas. But it was evident at a gathering of nearly two thousand scientists and clinicians last month* that there is still much to be done before such advantages can be translated into improved health care.

Many of the participants were already aware of the most recent advance, described at the meeting by F. Collins (University of Michigan) and D. Rich (University of Iowa), and published in September^{4,5} — correction of the abnormal chloride channel permeability in cultured CF cells by expression of normal cystic fibrosis transmembrane conductance regulator (CFTR). Collins, together with J. Riordan and L.-C. Tsui (Hospital for Sick Children, Toronto), presented research updates on the CFTR gene 'one year later'. The CFTR gene on chromosome 7 has 27 exons and three AP-1 sites upstream of the initiation site, such that transcription can be dramatically reduced by the addition of phorbol ester. The presence of DNase I hypersensitive sites flanking the first exon in T84 colon carcinoma cells, but not B cells, correlates with the observed tissue-specific expression of CFTR (Collins). There are several alternatively transcribed products of the CFTR gene, as seen using the polymerase chain reaction, which splice out exons encoding the first nucleotide-binding fold. In 1 per cent of cases, a protein with a different N terminus is predicted, but the functional significance of these CFTR splice variants remains unknown.

In common with many groups presenting data, Riordan and colleagues have been raising antibodies to CFTR peptides and fusion proteins, and studying the localization of CFTR protein. Preliminary results show that antibodies stain T84 cells and sweat glands, but not Panc-1 (pancreatic) cells. Two antibodies stain the reabsorptive duct (but not the secretory coil) of sweat glands, although it is not yet possible to be certain that the staining is confined exclusively to the apical border. Further studies with immunoelectron microscopy should unequivocally localize CFTR.

Another area of intense activity is the attempt to produce a murine model for CF by homologous recombination in embryonic stem cells. The complete mouse gene has now been cloned (P. Scambler, St Mary's Hospital, London) and the gene product reveals 75–80 per cent amino-acid similarity to the human

protein. Interestingly, all the amino acids affected in human CFTR mutations described to date are conserved in the murine sequence. No one has yet been successful in 'knocking out' exons of the CFTR gene; one reason seems to be the lack of innate recombination at this locus, which may be improved by including greater lengths of flanking non-coding or immunoglobulin sequences (K. Thomas, University of Utah).

Studies of the mammalian CFTR protein are therefore in their infancy, but research on homologues of CFTR, such as the yeast protein STE6, is more advanced (S. Michaelis, Johns Hopkins School of Medicine). STE6 is a 1,290-residue transmembrane protein which mediates the secretion of the farnesylated α -factor mating pheromone. Site-directed mutagenesis of two conserved residues in the first nucleotide-binding fold (NBF) results in loss of function. The analogous mutations in the second NBF have a much smaller effect (in agreement with the reduced number of human mutations discovered in the second half of the gene). Homology in the region corresponding to the common deleted phenylalanine residue (Δ F508), however, is not great, and substitutions of two possibly homologous amino acids in STE6 have not impaired function. STE6 is but one member of a transporter superfamily encompassing over 30 proteins, found in species from bacteria to humans, which pump single amino acids, peptides and a variety of other compounds. Understanding the substrate of CFTR remains one of the priorities in CF research.

After the discovery that Δ F508 consti-

tutes 70 per cent of CF mutations, it was hoped that the remaining 30 per cent or so would be accounted for by a few other changes. This has proved not to be the case. Over the past year, the 'CF genetics consortium' (which represents some 80 groups investigating the gene) has charted each new mutation as it has been uncovered. Last month the total had reached 61, of which 24 have been published or are 'in press' (see table). Many of these are rare 'private' mutations, occurring in individual families of all racial and ethnic backgrounds; but whereas over 80 per cent of Western European CF mutations have been identified, the figure is much lower (nearer 50 per cent) for US blacks (G. R. Cutting, Johns Hopkins School of Medicine).

Some mutations occur on 2–5 per cent of CF chromosomes, for example G551D (ref. 6), which is found with increased frequency in the British Isles and northern Europe. Three other mutations occur with roughly this frequency, although the mechanism for their relative prevalence differs. G542X is ubiquitous across populations, and is thought to be an old mutation that has spread by admixture. R560T is unusually common in Northern Ireland, and may have spread through a founder effect. Unlike other mutations, which have arisen on one haplotype, R553X has arisen on at least two different haplotypes in different communities (US blacks and in Northern Ireland), and is probably a recurring mutation at a CG dinucleotide (Cutting).

Of the mutations reported to the consortium, only two are within the large, cytoplasmic, putative regulatory region (R domain) that separates the two halves of CFTR (Tsui). There is also a second amino-acid deletion, at the residue preceding Phe 508. Most of the mutations identified so far map to the first half of the

Mutations identified in the cystic fibrosis transmembrane conductance regulator

Name	Amino-acid change	Nucleotide change	Exon	Reference
D110H	Asp → His (110)	G → C (460)	4	9
R117H	Arg → His (117)	G → A (482)	4	9
R347P	Arg → Pro (347)	C → G (1173)	7	9
A455E	Ala → Glu (455)	C → A (1496)	9	10
Q493X	Gln → Stop (493)	C → T (1609)	10	10
Δ I507	Ile deletion (506/7)	3 bp deletion	10	10
Δ F508	Phe deletion (508)	3 bp deletion	10	3
1717-1G → A	splice mutation	G → A (1717-1)	Intron 10	10
G542X	Gly → Stop (542)	G → T (1756)	11	10
S549N	Ser → Asn (549)	G → A (1778)	11	6
S549I	Ser → Ile (549)	G → T (1778)	11	10
S549R	Ser → Arg (549)	T → G (1779)	11	10
G551D	Gly → Asp (551)	G → A (1784)	11	6
R553X	Arg → Stop (553)	C → T (1789)	11	6
A559T	Ala → Thr (559)	G → A (1807)	11	6
R560T	Arg → Thr (560)	G → C (1811)	11	10
Y563N	Tyr → Asn (563)	T → A (1819)	12	10
P574H	Pro → His (574)	C → A (1853)	12	10
2566insAT	frameshift	AT insertion (2566)	13	12
W846X	Trp → Stop (846)	G → A (2670)	14a	11
Y913C	Tyr → Cys (913)	A → G (2870)	15	11
3659delC	frameshift	C deletion (3659)	19	10
S1255X	Ser → Stop (1255)	C → A (3896)	20	7
W1282X	Trp → Stop (1282)	G → A (3978)	20	11

* Fourth Annual North American and 1990 International Cystic Fibrosis Conference, Arlington, Virginia, 3–6 October 1990.

Compiled by L.-C. Tsui, cystic fibrosis genetics consortium.

protein, in particular the first NBF (exons 9–12). Surprisingly, for a gene of this size, no large-scale deletions or rearrangements have been documented.

There is much interest in correlating the genotype of affected individuals with phenotype (mild or severe disease, pancreas sufficiency or insufficiency). For example, $\Delta F508$ is a 'severe' mutation, whereas 'mild' mutations are found in exons 1, 9 and 12. Surprisingly, two pancreas sufficient patients have been identified out of 300 who are $\Delta F508$ homozygotes (E. Kerem, Hospital for Sick Children, Toronto) — changing diagnosis or the involvement of other genetic factors in modulating the phenotype are possible explanations. More remarkable still is the finding of rare patients with nonsense mutations and yet mild phenotype, which implies that complete absence of functional CFTR (or presence of a severely truncated form) is less of an impairment to cells than protein containing amino-acid substitutions (Cutting; see also refs 7 and 11).

The recent success in complementing the CF phenotype^{4,5} begs the question: what are the future prospects for gene therapy? Many seem to believe that delivering a suitable gene to the airway epithelial cells, and thereby correcting their phenotype, is achievable. J. Wilson (University of Michigan) indicated that new techniques for 'in vivo' retroviral delivery must be developed, but that recent success for vascular endothelial cells⁶ is encouraging. R. Crystal (NHLBI) eloquently discussed the advantages and drawbacks of using modified adenovirus to deliver normal copies of the CFTR gene to the affected airway cells. Some of their advantages over retroviruses include natural tropism for respiratory epithelium, and no requirement for host cell division. The disadvantages, including the unresolved question of safety, were touched on only briefly, and Crystal's summation that there is "no reason why we can't do gene therapy for cystic fibrosis" may have left some in the audience with the false impression that gene therapy is imminent. But no one, surely, could fail to echo the hope of Robert Beall that his organization, the US Cystic Fibrosis Foundation, would be "out of business" by the year 2000. □

Kevin Davies is an assistant editor of *Nature*.

Cooling flows and dark matter

Simon D. M. White

OVER the past decade, X-ray telescopes have produced many images of the hot and tenuous atmospheres that pervade rich clusters of galaxies. These pictures suggest that substantial amounts of intergalactic gas are cooling off and turning into condensed objects less massive and much less luminous than ordinary stars. If 'jupiters' are indeed forming in such cooling flows, it seems that these objects, rather than some new and exotic form of matter, may provide much, perhaps all, of the 'missing mass' whose gravitational effects induce the observable motions of galaxies in clusters and larger structures. New work by Thomas and Fabian¹ and Ashman and Carr² uses observed cooling flows as analogues of the process of galaxy formation. The theories are still sketchy, but they are causing heated debate as they require the sacrifice of many sacred cows of today's cosmology.

By the end of the 1970s, the dynamical evidence that large amounts of matter remain unseen in the galaxies was generally accepted by the astronomical community. Substellar objects, insufficiently massive to shine by nuclear fusion, were considered a plausible repository for this dark matter. In a popular galaxy-formation theory of the time, such jupiters formed in an unspecified manner at high redshift (in the early Universe), and then clustered into larger and larger dark halos. Residual gas condensed within these halos to make galaxies³. In the early 1980s this model became the 'cold dark matter' theory; jupiters were forgotten, and the dark mass was instead assumed to be an entirely new, nonbaryonic (non-nuclear) form of matter. The high-density Universe preferred by the inflationary model of the Big Bang could then be reconciled with the low baryon density required by the theory of Big Bang nucleosynthesis. Galaxies formed as before by the cooling of ordinary gas within an evolving hierarchy of exotic dark halos⁴. The theory still required unseen baryons, because observed stars or intergalactic gas provide considerably less than the minimum baryon density required for cosmic nucleosynthesis to work.

X-ray telescopes detected large amounts of hot gas in galaxy clusters in some of the first rocket-borne experiments. Such intergalactic gas seemed an attractive hiding place for the extra baryons. But gas near the middle of many clusters turns out to be radiating so strongly that it must cool off in a few billion (10^9) years. In so doing it should sink to the cluster centre, much as galaxies condense in the theories just discussed⁵. In some objects, hundreds of solar masses of gas

appear to be condensing every year. Despite this, there is almost no sign of the bright massive stars associated with most known regions of star formation. Cooling flows seem to produce predominantly dark stars, in other words, jupiters. This conclusion has been strongly resisted by many astronomers because neither the gas motions nor the jupiters can be observed directly. Nevertheless, a number of observations indicate that low-temperature gas is indeed present, and that condensed objects are forming at a substantial rate. Thus most of the missing baryons might be contained in a population of jupiters.

In their new work¹, Thomas and Fabian try to construct a galaxy-formation theory based directly on observations of cooling flows. Their fundamental hypothesis is that jupiters form only in situations where cooling is relatively slow, as in observed cluster flows, and that normal bright stars form when cooling is rapid. This leads them to the conclusion that galaxies were born in the early Universe, but that dark matter could form only later when more massive systems are present. If the Universe contains a dominant amount of non-baryonic dark matter, the authors show that this idea explains the observed amounts of iron in the hot gas in clusters, and can account for the conversion of many baryons into jupiters. However, the model is then a minor variant of the standard cold dark matter theory. In a more interesting case, the Universe contains only baryonic material. Thomas and Fabian show that this does not work so well because cooling flows occur only in very massive systems. As a result the model gives no explanation for origin of the dark matter which makes up the halos of our own, or other isolated galaxies.

Another problem with the hypothesis of Thomas and Fabian is discussed in the new paper² by Ashman and Carr. The amount of material processed through cooling flows is inferred to be quite small compared with that processed at earlier times in objects where cooling is rapid. This hardly seems promising if the goal is to end up with most of the baryons in the form of dark matter rather than visible stars. A possible way out has been known for fifteen years: in small systems, supernovae produced by the first few stars might blow away the remaining gas before it can condense⁶. Most of the gas might then survive until the epoch of cooling flows. Nevertheless, if jupiters make up the halos of ordinary galaxies, it seems that they must have condensed at early times when cooling was rapid. Something other than cooling speed must then distinguish the formation of jupiters from that

1. Rommens, J. M. *et al. Science* **245**, 1059–1065 (1989).
2. Riordan, J. *et al. Science* **245**, 1066–1073 (1989).
3. Kerem, B.-S. *et al. Science* **245**, 1073–1080 (1989).
4. Drumm, M. L. *et al. Cell* **62**, 1227–1233 (1990).
5. Rich, D. P. *et al. Nature* **347**, 358–363 (1990).
6. Cutting, G. R. *et al. Nature* **346**, 366–369 (1990).
7. Cutting, G. R. *et al. New Engl. J. Med.* (in the press).
8. Nabel, E. G., Plautz, G. & Nabel, G. J. *Science* **249**, 1285–1288 (1990).
9. Dean, M. *et al. Cell* **61**, 863–870 (1990).
10. Kerem, B.-S. *et al. Proc. natn. Acad. Sci. U.S.A.* (in the press).
11. Vidaud, M. *et al. Hum. Genet.* (in the press).
12. White, M. B. *et al. Nature* **344**, 665–667 (1990).

of ordinary stars. One possibility might be that ordinary stars form only in rotationally supported disks⁷. These are, after all, the only places where widespread star formation is currently observed. Perhaps star formation in other situations always produces jupiters.

Jupiters are an attractive candidate for the dark matter because it is intrinsically implausible to assume that most of the Universe is locked up in an entirely new and unknown form of matter. Although it seems very difficult to make a purely baryonic Universe consistent with the low baryon density required by nucleosynthesis, with the high mass required to explain the large-scale motions of galaxies, and with the extraordinary smoothness of the microwave background radiation, the task may not be hopeless. A very positive development has been the realization that if the halo of our own Galaxy is made of jupiters, they can be detected directly. Observable gravitational lensing occurs when one of them passes directly in front

of a more distant star⁸. Two groups are currently designing experiments to monitor a million or more stars in our nearest satellite galaxy, the Large Magellanic Cloud. Regular observation for a year or more is necessary. If successful, this work will test whether the dark halo of the Milky Way is made of objects more massive than planets. □

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1. Thomas, P.A. & Fabian, A.C. *Mon. Not. R. astr. Soc.* **246**, 156–162 (1990).
2. Ashman, K. & Carr, B.J. *Mon. Not. R. astr. Soc.* (in the press).
3. White, S.D.M. & Rees, M.J. *Mon. Not. R. astr. Soc.* **183**, 341–358 (1978).
4. Blumenthal, G.R., Faber, S.M., Primack, J.R. & Rees, M.J. *Nature* **311**, 517–525 (1984).
5. Fabian, A.C., Nulsen, P.E.J. & Canizares, C.R. *Nature* **310**, 733–740 (1984).
6. Larson, R.B. *Mon. Not. R. astr. Soc.* **169**, 229 (1974).
7. Toomre, A. in *The Evolution of Galaxies and Stellar Populations* (eds Tinsley, B.M. & Toomre, A.) 401 (Yale Univ. Obs., 1977).
8. Paczynski, B. 1986 *Astrophys. J.* **304**, 1–5 (1986).

PALAEONTOLOGY

Seeking the arthropods of Eden

W. D. Ian Rolfe

THE well-documented appearance and expansion of land vascular plants in the late Silurian and early Devonian (420–390 million years (Myr) ago) was thought recently to have been followed by a similar colonization of the land by early arthropods^{1,2}. This understanding was based on records of early millipede-like fossils and on three Devonian assemblages (at 395, 390 and 380 Myr ago) containing many groups of indisputable land arthropods. It was a satisfying story, but one which seems increasingly unlikely as new finds are made. One line of evidence comes

from early plant microfossils, which suggest that a quite different kind of land vegetation existed in Ordovician times (440 Myr ago), a vegetation which could easily have supported animal life^{3–5}. Many separate, often equivocal, pieces of information hint that these plant pioneers were not alone: Ordovician worms, late Ordovician burrows in a palaeosol from Pennsylvania, early Silurian arthropod fragments also from Pennsylvania, and late Silurian faecal pellets from Sweden, presumably produced by a fungivorous microarthropod⁶. Now, as reported by

Jeram and colleagues⁶ in last week's *Science*, a new assemblage of land arthropods has been found in the upper Silurian (411 Myr ago⁷) of Shropshire, England, which proves that the animal invasion of the land did indeed occur long before the Devonian.

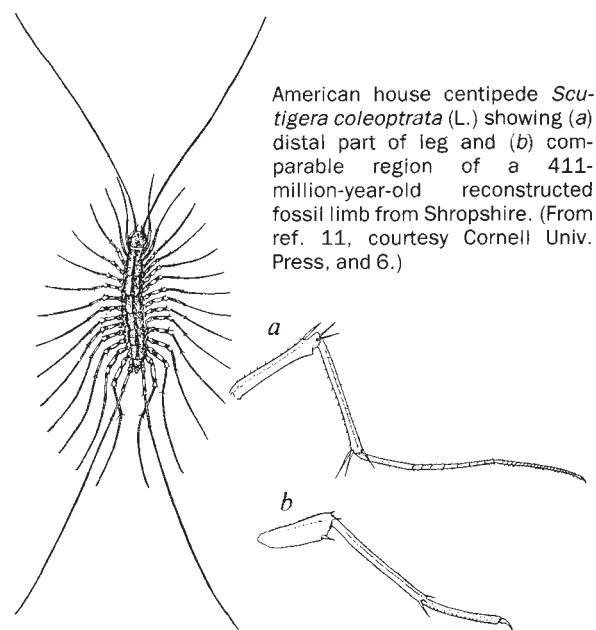
Like most of the previously known lower Palaeozoic terrestrial arthropod assemblages, this one was also found by serendipity, as a by-product of the systematic search for minute 'coalified' plant fossils. It comes from Ludlow, Shropshire, and occurs just above the famous Ludlow Bone Bed, which Sir Roderick Murchison aptly compared to "a heap of broken

beetles". Along with eurypterids, aquatic scorpions and other more usual fossils, there is a trigonotarbid, a spider-like arachnid that occurs in all three Devonian terrestrial faunas. Most of the cuticles, though, belong to kampecarids — superficially millipede-like forms long known from the Devonian Old Red Sandstone. They have recently been referred to a group of their own, and it has been suggested that they were aquatic⁸, but this is equivocal⁹.

Most intriguing of all are fragments of what may be two types of centipede. The sawblade-like limbs of these animals have also been recorded from the Devonian (380 Myr ago) of Gilboa in New York state, and referred to the *Scutigero-morpha*¹⁰. These present-day hunters of fast-moving prey were thought to be highly advanced, but it is now accepted that they are most similar to the probable common ancestor of all centipedes. Because this group includes the common US house centipede, it raises the question of whether these fossils are modern contaminants.

Jeram *et al.* exclude contamination because the fossils' alteration is consistent with the diagenetic history of the matrix; extinct and/or extopic taxa are present; the results are repeatable; and specimens were visible *in situ* before maceration. But they say that the recently described bristletail *Gaspea* from the Canadian Devonian fails to satisfy these criteria: the specimen is unique; its cuticle lacks impressions of sedimentary particles from the matrix; and the supposedly distinctive eye separation may only be an artefact. The authors therefore do not accept *Gaspea* as a genuine fossil, thus eliminating the earliest North American hexapod within two years of its description.

A further remarkable fact about the Ludlow assemblage is that, like the other early terrestrial assemblages of fossil arthropods, it is dominated by predators. Some accept this as a true reflection of the situation that existed, with arthropod carnivores and detritivores dominating land-animal communities for tens of millions of years before herbivores evolved¹¹. Others believe that the lack of herbivory indicates



American house centipede *Scutigera coleoptrata* (L.) showing (a) distal part of leg and (b) comparable region of a 411-million-year-old reconstructed fossil limb from Shropshire. (From ref. 11, courtesy Cornell Univ. Press, and 6.)

1. Shear, W.A. in *Short Course in Palaeontology* Vol. 3 (ed. Culver, S.J.) 197–213 (Paleontological Society, Tulsa, 1990).
2. Selden, A. & Edwards, D. in *Evolution and the Fossil Record* (eds Allen, K. & Briggs, D.E.G.) 122–152 (Belhaven Press, London, 1989).
3. Gray, J. (ed.) *Paleoecology* (Elsevier, Amsterdam, 1988).
4. Little, C. *The Terrestrial Invasion* (Cambridge Univ. Press, 1990).
5. Rolfe, W.D.I. *Phil. Trans. R. Soc.* **B309**, 207–218 (1985).
6. Jeram, A.J., Selden, P.A. & Edwards, D. *Science* **250**, 658–661 (1990).
7. Harland, W.B. *et al.* *A Geologic Time Scale 1989* (Cambridge Univ. Press, 1990).
8. Almond, J. *Phil. Trans. R. Soc.* **B309**, 227–237 (1985).
9. Shear, W.A. & Bonomo, P.M. *Am. Mus. Nov.* **2927**, 1–30 (1988).
10. Edwards, D., Fanning, U. & Richardson, J.B. *Nature* **323**, 438–440 (1986).
11. Snodgrass, R.E. *Textbook of Arthropod Anatomy* (Cornell Univ. Press, Ithaca, 1952).

a true soil fauna². A further contributory factor may be that herbivorous arthropods would be largely unsclerotized, and therefore unlikely to survive the acid-bath extraction that yielded most of the cuticles.

Palaeobotanists have transformed knowledge of early land plants and their evolution in recent decades, yet still they cry "We need more fossils"¹⁰. That is even truer of early land arthropods, but must

palaeozoologists continue to rely on the rich pickings of palaeobotanists for earlier finds? Several recent research grant proposals from fossil arthropod workers suggest not, and we can look forward to earlier Palaeozoic records as the search intensifies. □

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METEORITICS

Extraterrestrial amino acids and terrestrial life

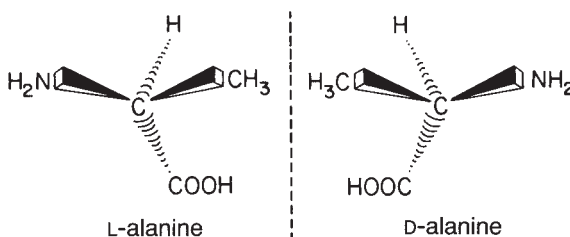
Christopher F. Chyba

SINCE the Swedish chemist Baron Jöns Jacob Berzelius first analysed the Alais meteorite for organic molecules¹ in 1834, attempts to forge a link between extraterrestrial organic materials and terrestrial life have remained alluring, but often deceptive. Two distinct investigations are demanded in exploring such links: accurately characterizing the organics present in extraterrestrial bodies; and evaluating possible mechanisms for delivering these molecules intact to Earth. New studies reported in this and last week's issues^{2,3} hold the promise of important advances in both endeavours. The results of Engel *et al.*², if upheld, would suggest that the characteristic 'handedness' of biochemistry on Earth may ultimately have been determined by an asymmetry already existing in extraterrestrial amino acids. The paper on page 157 of this issue³ by Zahnle and Grinspoon presents one mechanism, the gentle collection of organic-rich dust evolved from an evaporating comet, whereby amino acids might in fact reach the Earth without being destroyed. A similar suggestion has been made previously in the prebiotic context⁴, but Zahnle and Grinspoon directly confront the most important datum currently available to test such hypotheses: the apparently extraterrestrial amino acids discovered⁵ in abundance in sediments at Stevns Klint, Denmark, marking the Cretaceous-Tertiary (K/T) boundary, the stratum associated with the major extinction 65 million years ago.

Murchison meteorite

The first identifications of amino acids in meteorites failed to survive doubts concerning terrestrial contamination, and were made in the midst of an acrimonious controversy over claimed observations of microfossils and biogenic organic molecules in meteorites⁶. In 1970, Kvenvolden *et al.*⁷ published the first of a series of studies which unequivocally demonstrated that amino acids in the Murchison meteorite, which had fallen the previous year,

were extraterrestrial and probably abiogenic. The evidence was threefold: Murchison's amino acids were nearly racemic (equal) mixtures of the D and L enantiomers (see the figure), whereas terrestrial organisms use almost exclusively the L enantiomer; extractable Murchison



The L and D enantiomers (from the Greek *enantios*: opposite) of the amino acid alanine. The two molecules are mirror images; no rotation through three dimensions can superimpose them. The enantiomers rotate the plane of polarization of light in opposite directions; unequal mixtures are therefore optically active. A mixture consisting of equal numbers of each is said to be racemic. Terrestrial life uses almost exclusively the L-amino-acid enantiomers.

organic compounds had $\delta^{13}\text{C}$ values (which measure the carbon isotope ratio $^{13}\text{C}/^{12}\text{C}$) that were greater than those in any naturally occurring terrestrial organics; and Murchison contained non-biological amino acids — those not among the 26 or so commonly found in terrestrial organisms. At least 74 amino acids have now been identified in Murchison extract⁸.

There is no difficulty in the non-destructive delivery of organic molecules to Earth in objects as small as the Murchison fragments. The bulk of the mass in potential terrestrial impactors, however, is concentrated in much larger bodies. For extraterrestrial material to have had a substantial influence on prebiotic chemistry, it would seem that this reservoir must somehow be tapped. Yet even objects as small as 100 metres in radius cannot be sufficiently decelerated by the Earth's present atmosphere for organic inclusions to survive the ensuing violent impact⁹.

This is one reason why the reported⁵ high abundance of the presumably extra-

terrestrial amino acids α -amino-isobutyric acid (AIB) and isovaline at the K/T boundary is so remarkable. (AIB and isovaline are extremely rare in the biosphere, but common in meteorites; moreover, the K/T isovaline is racemic.) A cosmochemical interpretation of the iridium (Ir) abundance at the boundary implies that it was created by an impactor with a diameter of 10 km; such an object would hit the Earth with a kinetic energy equivalent to 10^8 megatons of explosive, enough to incinerate even the hardest organics⁹. Yet the AIB/Ir ratio at Stevns Klint is substantially higher than for Murchison. More puzzling still, the amino acids were found tens of centimetres above and below the K/T boundary, but not in the boundary clay itself.

Assuming that these data are not anomalous, how are they to be explained? Zahnle and Grinspoon take the sedimentary record at face value, and suggest that Earth swept up the amino acids "gently and non-destructively" in cometary dust over a period of around 10^5 years. Organic molecules in interplanetary dust particles (IDPs) reach the Earth today⁴; a sufficiently large comet trapped in the inner Solar System could have provided the high IDP flux needed to deposit the amino acids around the K/T boundary. The boundary clay itself would then record the impact of a fragment of the parent comet, an impact which amino acids did not survive.

Zahnle and Grinspoon cite a report by D. Carlisle of amino acids found within, rather than around, the boundary clay at a Canadian K/T site. These results apparently corroborate the existence of K/T amino acids; it will be important to know if Stevns Klint AIB/Ir ratios are typical or anomalously high. Only the depositional history of the Canadian site, combined with detailed knowledge of the amino acid distribution, will show whether these results are consistent with the comet-dust model.

A difficulty that has received too little attention in all IDP organic-delivery schemes is the question of whether IDP atmospheric deceleration is really gentle enough for amino acids to survive. Models predict cometary IDPs to be typically heated to around 900 K for 5–15 seconds during atmospheric entry¹⁰. A naive application of thermal decomposition rates for amino acids⁹ suggests these molecules would survive around 800 K for 1 s. But the sole data available are for decomposition in solution, and these rates are of dubious relevance to degradation of amino acids in a mineral matrix. Laboratory rate parameters for a variety of matrices are badly needed to resolve this

question. In any case, the harder organics⁹, after some thermal processing, will certainly survive, and one is free to speculate³ that surviving amino acid precursors may then be synthesized into the observed products.

One apparent problem with the comet-dust model may be less severe than has been suggested³. Data collected by R. Rocchia *et al.*, cited by Officer and Drake¹¹, show that Ir values at Stevns Klint are correlated with the clay fraction; expressed on a carbonate-free basis, Ir concentrations stay within a factor of two of the peak clay abundance out to 30–40 cm above and below the boundary, and remain above background levels out to around 50 cm. These distances correspond to 10^4 – 10^5 -year depositional times, and suggest that the putative comet dust was delivering Ir as well as amino acids throughout its accretion. (However, high AIB/Ir levels in the dust still appear to be required.) I emphasize that such long-term cometary depositional histories rebut the argument¹¹ that claimed 10^4 – 10^5 -year timescales for K/T Ir deposition necessarily favours explanations of the K/T catastrophe in terms of extended terrestrial volcanism.

In any case, altogether different explanations for the K/T amino acids are possible. Extensive experimental data show that amino acids can be synthesized by quenched shock heating of reducing gases¹². It is commonly asserted that such recombination could not have occurred for vaporized impactor material in an O_2 -rich background atmosphere^{3,5}. In fact, production efficiency remains high even in background air¹², so post-impact shock synthesis is not yet ruled out⁹. A final possibility is organic delivery following catastrophic impactor fragmentation. A 10-km diameter bolide would be too large to be affected by the atmosphere, but if several smaller objects or fragments were responsible for the K/T geochemical signatures, each might have airburst in the atmosphere before hitting the ground. A carbonaceous chondrite did exactly this over Revelstoke, Canada, in 1965; photomicrographs of the millimetre-sized fragments recovered reveal unheated interiors¹³ within which organics should have survived⁹.

Significant sources

Our preliminary quantitative work¹⁴ suggests that IDPs⁴, airbursts and, possibly, quench syntheses, could have been significant sources of prebiotic organics on early Earth. In the first two cases, the report last week by Engel *et al.*² of an excess of L-type alanine in Murchison is potentially of great importance. No convincing explanation yet exists for why terrestrial life uses L, not D, amino acids. Abiotic explanations must either account for L/D ratios which differ from unity by

more than statistical fluctuations, or provide an amplification mechanism that proceeds faster than natural racemization. Neither programme has yet been successful¹⁵, and the possibility that the predominance of L-forms is simply an accident of early biology remains a leading alternative. Finding a meteoritic preference for L-amino acids would 'explain' terrestrial biology's choice by pushing the problem out into the cosmos — or it might imply yet-undiscovered abiotic processes that could also have operated on the early Earth.

If the data of Engel *et al.* are valid, there is a third possibility that must be forthrightly addressed. Non-racemic mixtures of amino acids have long been suggested as indicators of biologically derived molecules¹⁶. Entire papers at 'exobiology' meetings have been devoted to instrumentation designed to detect optical activity. Does not scientific honesty then require us to consider the new results as *prima facie* evidence of extraterrestrial life?

Scientific humility answers no. A simple failure to find an abiotic mechanism cannot in itself require an appeal to biology. (Indeed, A. Salam and J. Strathdee (personal communication) have just proposed a low-temperature quantum amplification of the unequal ground-state energies — around 10^{-19} electron volts, due to parity-violating weak nuclear interactions — of L and D amino acid enantiomers, which they argue could create non-racemic mixtures.) Results from the Viking biology packages on Mars are powerful reminders that prejudices about supposedly unambiguous attributes of terrestrial biology may crumble in the face of unanticipated extraterrestrial chemistry. In any case, the complete structural diversity of the Murchison amino acids argues for non-catalytic, thermodynamically-controlled (that is, abiotic) synthesis⁴.

Any detection of non-racemic amino acid mixtures must exclude the possibility of contamination due to L-rich terrestrial microorganisms. This concern was raised over an earlier report of non-racemic Murchison amino acids; it is especially worrying that only protein amino acids have been claimed to be non-racemic, and that it is the L enantiomer of alanine that is found in excess¹⁷. Both these results are, of course, consistent with contamination. The key result of Engel *et al.*² is that the L and D enantiomers of alanine in Murchison have extraterrestrial $\delta^{13}C$ values of +27 and +30 parts per thousand (‰), respectively. A putative terrestrial alanine contaminant, they show, would need an isotopic composition of +10 ‰ to arrive at the observed L-alanine bulk value, and the authors argue that no such terrestrial sources are apparent. (Terrestrial inorganic carbon has $\delta^{13}C$ 0‰; microorganisms typically fractionate carbon

isotopes to lighter values, producing organics with $\delta^{13}C$ as low as –40‰.)

Unfortunately, at least one plausible such contaminant does exist. It has recently been shown¹⁸ that a wide variety of common bacteria are able to obtain their carbon and energy needs from abiotically produced 'tholins', laboratory materials that provide good analogues to meteoritic and cometary organics⁹. This raises the possibility of contamination of Murchison by terrestrial bacteria that consume ^{13}C -rich organics, then produce $\delta^{13}C$ -rich amino acids. Methane from Murchison, for example, has $\delta^{13}C$ = +9.2‰, and certain other organics have higher values⁸. Moreover, at least one bacterium, *Methanobacterium thermoautotrophicum*, fractionates (admittedly under special circumstances) carbon to heavier isotopes¹⁹, by as much as +13 ‰. A bacterium capable of such fractionation while metabolizing, say, the Murchison benzene-methanol extract (which includes⁸ nonvolatile aromatics and the higher alkanes, and has $\delta^{13}C$ = +5 ‰), could produce the required effect.

Unanticipated chemistry and uncertain contamination have been the bane of 'exobiology'. Occam's razor, the nineteenth century name given to various versions of William of Ockham's fourteenth century maxim, remains a useful warning. The actual formulation was "*pluralitas non est ponenda sine necessitate*": plurality is not to be posited without necessity. This was meant as a methodological, not an ontological, admonition²⁰. Understood in this way, it puts the pursuit of familiar explanations before that of more encompassing ones: the former must be excluded before we may embrace the latter. □

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- Berzelius, J.J. *Ann. phys. Chem.* **33**, 113–148 (1834).
- Engel, M.H., Macko, S.A. & Silfer, J.A. *Nature* **348**, 47–49 (1990).
- Zahnle, K. & Grinspoon, D. *Nature* **348**, 157–160 (1990).
- Anders, E. *Nature* **342**, 255–257 (1989).
- Zhao, M. & Bada, J.L. *Nature* **339**, 463–465 (1989).
- Urey, H.C. *Science* **151**, 157–166 (1966).
- Kvenvolden, K. *et al.* *Nature* **228**, 923–926 (1970).
- Cronin, J.R., Pizzarello, S. & Cruikshank, D.P. in *Meteorites and the Early Solar System* (eds Kerridge, J.F. & Matthews, M.S.) 819–857 (University of Arizona Press, 1988).
- Chyba, C.F., Thomas, P.J., Brookshaw, L. & Sagan, C. *Science* **249**, 336–373 (1990).
- Flynn, G.J. *Icarus* **77**, 287–310 (1989).
- Officer, C.B. & Drake, C.L. *Science* **227**, 1161–1167 (1985).
- Barak, I. & Bar-Nun, A. *Origin Life* **6**, 483–506 (1975).
- Folinsbee, R.E., Douglas, J.A.V. & Maxwell, J.A. *Geochim. Cosmochim. Acta* **31**, 1625–1635 (1967).
- Chyba, C. & Sagan, C. *Bull. Am. astr. Soc.* **22**, 1097 (1990).
- Bonner, W.A., Blair, N.E. & Dirbas, F.M. *Origin Life* **11**, 119–134 (1981).
- Lederberg, J. *Nature* **207**, 9–13 (1965).
- Bada, J.L. *et al.* *Nature* **301**, 494–497 (1983).
- Stoker, C.R. *et al.* *Icarus* **85**, 241–256 (1990).
- Fuchs, G., Thauer, R., Ziegler, H. & Stichler, W. *Arch. Microbiol.* **120**, 135–139 (1979).
- Thorburn, W.M. *Mind* **27**, 345–353 (1918).

Knowing where you're going

S. J. Judge

MOTION of an observer creates 'optic flow' of the retinal image, containing information both about the observer's motion (or rather the motion of his eye) and about the three-dimensional structure of the world, as Gibson¹ was the first to emphasize. Although optic flow is purely a matter of geometry, recovering information from it is a non-trivial computational problem (see ref. 2 for a readable introduction). Although there are many theoretical schemes for analysing the phenomenon, it is not clear which computations the brain actually employs. For example, it is disputed³ whether the brain computes the differential invariants of the optic flow, which in theory provide the information needed. Now, on page 160 of this issue⁴, the neurophysiologists Roy and Wurtz report a remarkable new finding which bears on these questions.

The authors have been studying the activity of nerve cells in a zone of the cortex known as MST, once thought to be part of 'association cortex', but now known to be one of several areas specialized for the processing of visual motion signals. Individual MST cells are often direction-selective in their response to visual stimulation: movement in a given direction activates a cell, whereas movement in the opposite direction does not. The novel result is that 40 per cent of the MST cells studied by Roy and Wurtz have a further property: the direction of motion that activates these cells is dependent on whether the stimuli are closer to or further away from the animal than the fixation point (the target at which it is looking). If the cell is activated by stimuli in the foreground moving to the left, stimuli in the background will excite the cell if they move to the right.

Rather than using real three-dimensional targets, Roy and Wurtz simulated the effect of target distance using a stereoscopic display: the stimulus for one eye was projected onto a screen in red, and that for the other eye in green, and red-green goggles were used to separate the two images. This arrangement allowed the binocular disparity (the difference between the angular position of equivalent elements of the stimuli in the two eyes) to be manipulated independently of other changes that would accompany alteration of the distance of real targets.

One circumstance that would generate this pattern of visual stimulation is motion of the observer to one side as he fixates an object, or part of an object, in a scene with objects at different distances. Roy and Wurtz suggest that the disparity-dependent direction-selective (DDDS) cells might be part of a mechanism for

inferring the direction of motion of the organism through the environment from its visual consequences. Because the DDDS cells tend to prefer horizontal motion, they could supply information about the sense (leftward or rightward) of the side-to-side component of self-motion. Other cells in MST are already known to respond to the radial expansion that is characteristic of motion in the direction of the line of sight, so one has the beginnings, perhaps, of a coordinate system for analysing self-motion.

One might then ask whether the DDDS cells could provide a signal not only of the sense of self-motion but also its magnitude. Consider an observer moving at velocity v parallel to a plane with disparity d relative to the fixation point, and looking at right angles to the direction of motion (the conditions corresponding to those used in Roy and Wurtz's experiments). Then the angular velocity, m , of motion of the foveal retinal image of features on the plane, is given by $m = vd/I$, where I is the interocular separation. In particular, the ratio m/d is proportional to observer velocity, v , regardless of viewing

SYNTHETIC CHEMISTRY

distance. (This is a consequence of the well-known close geometrical relationship between stereopsis and motion parallax.) So the response of velocity-signalling DDDS cells should be invariant for stimuli with equal m/d , but vary systematically with m/d . From the examples of DDDS cells in Roy and Wurtz's report it is not clear whether this is the case. But it is clear that the two DDDS cells illustrated could only signal self velocity for scenes restricted to disparities less than a degree or so, as the cells' responses saturated at disparities of about one degree, for stimuli of constant m and varying disparity.

There are other circumstances that generate optic flow of the type to which DDDS cells are sensitive — such as the motion of an object, extended in depth, past an observer who is tracking a particular point on the object. Might it be that DDDS cells also play some role in the analysis of optic flow associated with object motion? □

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1. Gibson, J. J. *The Perception of the Visual World* (Houghton Mifflin, Boston, 1950).

2. Koenderink, J. J. *Vision Res.* **26**, 161–180 (1986).

3. Warren, W. H. & Hannon, D. J. *Nature* **336**, 162–163 (1988).

4. Roy, J.-P. & Wurtz, R. H. *Nature* **348**, 160–162 (1990).

Boron's molecular gymnastics

Keith Smith

BORON has frequently occupied an important position at the forefront of chemical development. For example, diborane (B_2H_6 ; compound **1**, Fig. 1), which resembles ethane (C_2H_6) in its formula but possesses two fewer electrons, for many years confounded attempts to rationalize its structure in terms of the electronic theory of bonding. The eventual resolution of the conundrum led to an appreciation of multicentre bonding. More recently, polyhedral boron compounds typified by pentaborane(9) (compound **2**, Fig. 2) were instrumental in the emergence of a general theory of cluster species^{1,2}. Boron has also provided one of the most widely used reducing agents, sodium borohydride, and a Nobel-prize-

winning synthetic reaction, hydroboration³. Now, new work in Germany⁴ illustrates how the propensity of boron compounds to take part in rearrangement reactions can lead to contortions on a molecular level which on a human level would make the most daring Soviet gymnasts gasp in disbelief.

The work in question concerns reactions of tri-*tert*-butylazadiboriridine (compound **3**, Fig. 2) with carbon monoxide. Peter Paetzold and his collaborators bubbled carbon monoxide through a solution of **3** at low temperature, whereupon crystals of compound **4** separated from the solution in high yield (Fig. 2a). The structure of compound **4** was confirmed by its nuclear magnetic resonance and mass spectra, and by X-ray crystallography. It possesses a nearly planar central six-membered ring with two three-membered rings joined in a spirocyclic fashion perpendicular to it.

When the carbon monoxide for the reaction was provided by photodecomposition of iron pentacarbonyl, an even stranger rearrangement took place producing compound **5**,

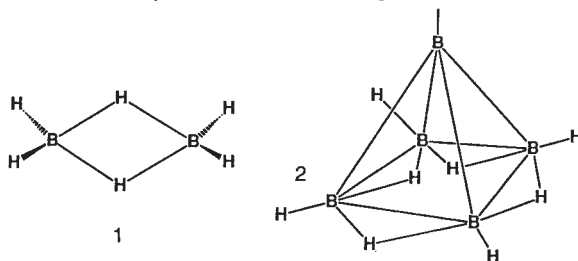


FIG. 1 Diborane (compound **1**) and pentaborane(9) (compound **2**); forerunners in chemical developments.

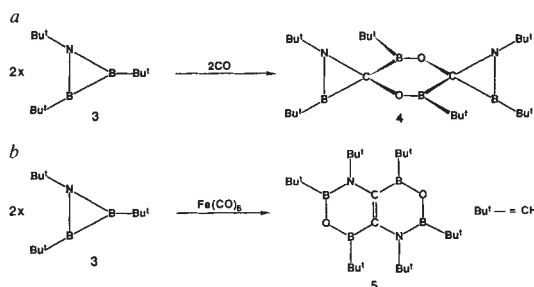


FIG. 2 Reactions of tri-*tert*-butylazadiboriridine (compound 3) with carbon monoxide discovered by Paetzold *et al.*⁴. *a*, With CO gas, in solution in pentane at dry-ice temperature for 30 min, forming compound 4. *b*, With CO produced from iron pentacarbonyl (Fe(CO)₅) by ultraviolet irradiation, at -30°C for 24 hours, yielding compound 5.

an isomer of 4 (Fig. 2b). In this case the product has a bicyclic structure resembling naphthalene but the central group of ten atoms do not form a fully planar arrangement. Rather, the central six atoms are in a single plane with the outer pairs (B–O) being displaced above and below the plane of the central atoms. The structure was again confirmed by X-ray crystallography and spectral analysis.

The structures of these products — which bear little resemblance to the reagents, let alone one another — clearly took Paetzold and his colleagues by surprise. Indeed, the fact that carbon monoxide reacts at all with compound 3 contrasts strongly with its inert behaviour towards the non-cyclic analogue of 3, Bu^t(Me₂N)B–B(NMe₂)Bu^t. How, then are the reactions to be understood?

First, three-coordinate boron compounds have only six electrons in the outer shell of the boron atom and are thus usually electrophilic. Direct attachment of two boron atoms to each other creates a situation in which both are in competition for any available electron density, and compounds containing such an arrangement of atoms could therefore be described as super-electrophilic. Such compounds are rarely stable unless the groups attached to the boron atoms are able to contribute some charge, as (for example) dimethyl-amino groups can. In the case of compound 3 there is only one nitrogen atom to donate a pair of electrons to two electron-seeking boron atoms and the structure is also strained owing to the three-membered ring. Thus, although the compound may be formally aromatic (having 2 π -electrons in a continuous ring), it is not too surprising that it is also highly reactive.

A clue to the reaction taking place (Fig. 2a) can be gained from reactions of carbon monoxide with simple trialkylboranes⁵ (Fig. 3). In such reactions carbon monoxide 'coordinates' to the trialkylborane (com-

pound 6) to give a species (compound 7) which can rearrange to an acylborane (compound 8). This is unstable and dimerizes and rearranges further to give a dioxadiborinane (compound 9). The production of compound 9 from compound 3 could take place by an entirely analogous sequence involving cleavage of the B–B bond during the first rearrangement step.

The formation of compound 5 (Fig. 2b) is more difficult to explain, involving as it does

the complete separation of the carbon and oxygen atoms of the original carbon monoxide. The German researchers offer no suggestion as to how it may be formed. However, the long reaction time and the irradiation with ultraviolet light are likely to be significant. Future experiments should be designed to establish whether

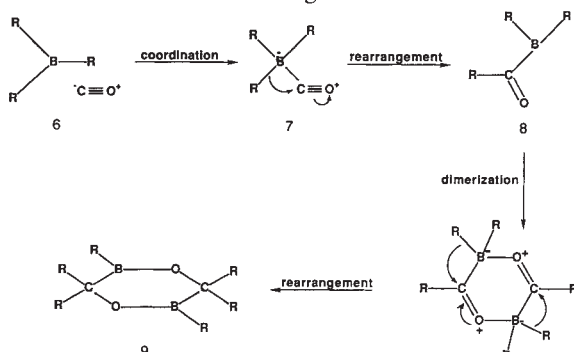


FIG. 3 The reactions of trialkylboranes (compound 6; R is an alkyl group) with carbon monoxide involve several rearrangement steps (curly arrows correspond to movement of electrons): a clue to the mechanism in the reactions discovered by Paetzold *et al.*⁴?

compound 4 rearranges into compound 5 on irradiation or whether an intermediate in the production of 4 can be diverted to 5 by irradiation.

Whatever the precise mechanisms of reactions such as these may be, the ability to create complicated molecular gymnastics in such apparently simple ways offers opportunities for the design of new methods which will reduce the number of synthetic steps needed to build up complicated structures. Even more exciting is the prospect of formation of new types of chemicals with properties as yet unimagined. □

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1. Wade, K. *Adv. inorg. Chem. Radiochem.* **18**, 1–66 (1976).
2. Williams, R.E. *Adv. inorg. Chem. Radiochem.* **18**, 67–142 (1976).
3. Brown, H.C. *Hydroboration* 2nd edn (Benjamin/Cummings, Reading, Massachusetts, 1980).
4. Paetzold, P., Redenz-Stormanns, B. & Boese, R. *Angew. Chem. Int. Ed. Eng.* **29**, 900–902 (1990).
5. Peitler, A., Smith, K. & Brown, H.C. *Borane Reagents* (Academic, London, 1988).

Cloud straining

KITE flying is one of the most pleasant of pastimes. Only modest skill is needed to manipulate the control lines of the kite and keep it aloft despite the gusts and fluctuations of the wind. A DREADCO engineer, more or less for fun, has recently automated this skill. Sensors on his 'autokite', coupled via its control cables to a computerized manipulator on the ground, enable it to be flown aloft and stabilized at any altitude without human intervention. No matter how the wind fluctuates, the autokite reacts to maintain its height.

Daedalus is now wondering how to exploit this neat invention. A permanent and stable kite would, of course, make a useful radio antenna or a platform for aerial photography. A large array of them, flown on command, might carry aloft an extended wire fence as an instantly erected protection against cruise missiles. But the most intriguing use, he reckons, is in rain making. Several projects round the world are studying the use of 'fog nets' to extract liquid water from mountain-top clouds. As the fog blows through the fine nylon mesh, some of the suspended droplets strike the fibres; a steady stream of water trickles down the net and can be collected. A square metre of such a net can trap 3 litres of water a day. If the net could be deployed in the sky, it could extract water from those tantalizing clouds that so often blow by without depositing rain.

So Daedalus is designing a really big 'flying net' to intercept the clouds. A long array of autokites will suspend a huge extended fog net between them. With proper design, the wind blowing through the net will generate enough lift to keep it up, so the autokites will merely have to keep it properly extended and positioned. Ground commands will lift the massive web to the right altitude for the local clouds, which will blow through it and deposit much of their water. This could be allowed to spill out of the bottom of the net in intense sprays of local rain; but a better arrangement would channel it down pipes in the control-cables for collection on the ground.

Thus our infuriating dependence on the weather would almost be eliminated. Even in rainy Britain, droughts often occur; and very little of the rain that does fall is usefully collected. But arrays of cloud-straining autokites will capture water all the time — except on those rare and perfect days of cloudless blue. And all of it will be collected; for storage by a private owner, or distribution through the public water-system. Cloud-fresh natural rainwater, unhardened by chalk and unsullied by lead pipes, should be pure enough for all domestic needs. It might even steal the market from those pretentious natural mineral waters.

David Jones

Parasites in *Drosophila* embryos

SIR—We wish to alert people studying early embryonic development in the fruit-fly *Drosophila melanogaster* of the possible presence of commensal parasites in some stocks similar to those described in *D. simulans* by S.L.O'N. and T.L.K. on page 178 of this issue¹. Although these stocks appear to grow normally, clusters of DAPI-stainable spots can be seen in their early embryos. We have observed these organisms in some wild-type *D. melanogaster* embryos when centrosomes

embryos because they can be seen easily only in very early or in growth-arrested embryos. Most previous studies of embryogenesis have focused on syncytial or later stages, in which the many *Drosophila* nuclei make the much fainter fluorescence of the parasites hard to see. By the blastoderm stage the parasites are no longer easily detectable in the somatic portion of embryos, although they can still be seen in pole cells¹. Endosymbionts can have profound effects on early develop-

Unique historical serendipity

SIR—Solomon H. Snyder in his News and Views article¹ *Planning for Serendipity* says "By the late 1930s, the chemical structure of Δ^9 -THC was sufficiently well known for drug companies to be able to synthesize analogues. . .".

In our paper², published in 1940, we reported the first isolation of an active molecule from the marijuana plant. Only in the late 1940s did R. Adams in the United States and A. Todd in Britain identify the psychomimetic molecule in marijuana as tetrahydrocannabinol (THC). Adams' synthetic Δ^1 -THC was found not to be the active agent during the 1960s. Synthesis of the active ingredient did not occur until 1967.

It would indeed have been a rude awakening for my colleagues and I at Caltech to find, after one year's research, that the active component had not only been isolated but synthesized, a unique historical case of serendipity.

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SNYDER REPLIES—Wawra is correct that Δ^9 -THC was not known in those days. My statement was that the general THC structure had been reasonably clarified by 1940. Indeed, Roger Adams had isolated a mixture of tetrahydrocannabinols in sufficient purity to administer them in a controlled study to prison volunteers on New York's Welfare Island, obtaining clear-cut psychoactive effects³, as part of Mayor LaGuardia's marijuana commission.

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1. Snyder, S.H. *Nature* **346**, 508 (1990).
2. Haagen-Smit, A.J. *et al. Science* **91**, 602–603 (1940).
3. Adams, R. *Bull. N.Y. Acad. Med.* **18**, 705 (1942).

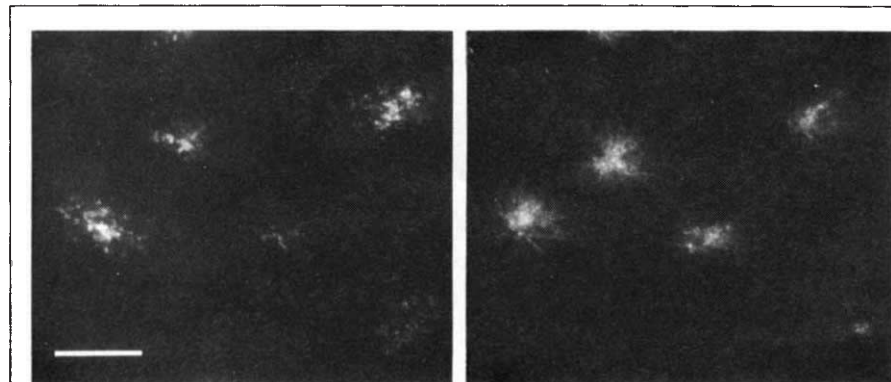
Alzheimer's *et al.*

SIR—We applaud St George Hyslop *et al.* (*Nature* **347**, 194–197; 1990) for publishing their extensive data, disappointing as they may be for those who had hoped that Alzheimer's disease might turn out to be genetically homogeneous. But we wonder if 56 authors are warranted. With the human genome project under way as a collaborative endeavour, it seems to us reasonable that a single article should not have more authors than we, as a species, have chromosomes.

FREDERICK HECHT
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Commensal parasites associated with asters in a *D. melanogaster* embryo. The left-hand panel (DNA) is DAPI-stained. The parasites are visible as dots clustered around the positions of asters (right-hand panel, stained with anti-tubulin antibodies; ref. 6 and references therein) and centrosomes (data not shown). The photograph shows a portion of a 0–2-hour-old embryo produced by a *fs* (1) *Ya*² mother^{4,6} and an *ms* (3) *k8l* father⁵. The embryo is arrested at pro-nuclear fusion, but the pronuclei are not located in the portion of the embryo shown in this photograph. Scale bar, 10 μ m.

have dissociated from nuclei following the inhibition of DNA replication with aphidicolin². In such cases, they are clustered around the centrosomes with a 'starburst'-like appearance (J.R. and D.M.G., unpublished data). Such starbursts can also be seen around free centrosomes and asters in some stocks in which nuclear division is blocked by mutation⁶ (H.L. and M.F.W., unpublished data; see figure). The starbursts can also cluster around the poles of mitotic spindles. This apparent association of starbursts with the mitotic apparatus is intriguing, and suggests an adaptation that would assist their transmission.

Commensal parasites can be eliminated by growing flies for one generation on 0.025% tetracycline followed by growth on tetracycline-free medium for a further generation to allow the flies to recover full fertility¹ or for one generation on 0.25 μ g per ml tetracycline followed by 5 days on 1 mg per ml tetracycline⁶. Care should be taken as these treatments may not permanently remove the parasites from the stock (T.L.K. and S.L.O'N.; M. Turelli, personal communication).

The commensal microorganisms may correspond to the maternally inherited commensal parasites reported by Wolstenholme³. We suspect that they have not been previously observed in DAPI-stained

ment in certain crosses of *D. simulans* or of other insects (see ref. 1), but we do not know whether the parasites we have observed would cause similar effects in *D. melanogaster*. In addition to their interference with phenotypic analysis, the possibility that the parasites may exert other effects should be kept in mind.

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1. O'Neill, S. L. & Karr, T. L. *Nature* **348**, 178–180 (1990).
2. Raff, J. & Glover, D. M. *J. Cell Biol.* **107**, 2009–2019 (1988).
3. Wolstenholme, D. R. *Genetics* **52**, 949–975 (1965).
4. Young, M.W. & Judd, B.H. *Genetics* **88**, 723–742 (1978).
5. Fuyama, Y. *Genetics* **112**, 237–248 (1986).
6. Lin, H. & Wolfner, M.F. *Cell* (in the press).

Oxygen in the Arabian Sea

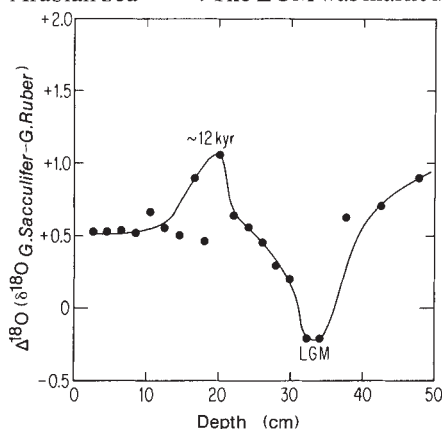
SIR—Variations in the oxygen isotope content ($\delta^{18}\text{O}$) of planktonic foraminifera from the Arabian Sea have been cited as evidence for a stronger northeast monsoon current at the last glacial maximum (LGM), about 18 kyr ago, than during the present day¹. Only by mapping spatial patterns of the detailed isotope and faunal variations can one truly resolve the salinity and temperature controls of foraminiferal tests in this region, but I suggest that the existing data support an alternative interpretation.

The northern Bay of Bengal receives a freshwater influx of $1.5 \times 10^3 \text{ km}^3 \text{ yr}^{-1}$ (ref. 2), whereas several smaller rivers drain only $0.3 \times 10^3 \text{ km}^3 \text{ yr}^{-1}$ into the southern Bay of Bengal³. Thus the Bay of Bengal is less saline than the Arabian Sea, and also results in a north-south salinity gradient. Extensive palaeo-oceanographic studies suggest that the LGM was marked by a weak Indian southwest monsoon^{2,4,5}. As the salinity of the Bay of Bengal is linked directly to the southwest monsoon-driven freshwater runoff, it is likely that salinities were similar in both seas during the LGM. Moreover, the combined runoff from the rivers fed by the northeast monsoon is much less than that of the Himalayan rivers. Therefore the reported¹ $\delta^{18}\text{O}$ shift at the LGM could be due to the changes in the surface conditions at the core locality.

The ecological constraints on the species *G. sacculifer* and *G. ruber* are known in greater detail⁶. Based on their modern distribution pattern in the Bay of Bengal, it can be argued that *G. sacculifer* abundance peaks during late summer and early autumn, and *G. ruber* during the summer⁷. A similar pattern seems to exist in the Gulf of Aden on the western Arabian Sea⁸. One can evaluate the Holocene surface conditions using the top three samples, covering the past 7–8 kyr. One finds average values of -1.59‰ and -2.11‰ for $\delta^{18}\text{O}_{\text{sac}}$ and $\delta^{18}\text{O}_{\text{rub}}$, respectively. From the known relationships between temperature, salinity and $\delta^{18}\text{O}$ for this region⁶, $\delta^{18}\text{O}_{\text{sac}}$ and $\delta^{18}\text{O}_{\text{rub}}$ are predicted to be -3‰ and -2.99‰ , respectively. Thus there are enrichments of 1.41‰ for *G. sacculifer* and 0.88‰ for *G. ruber*. But Duplessy *et al.*⁶ have noted depletions from equilibrium values of 0.6‰ for *G. sacculifer* and 0.7‰ for *G. ruber*; so $\delta^{18}\text{O}_{\text{rub}}$ can still be taken to represent surface conditions, but $\delta^{18}\text{O}_{\text{sac}}$ has an unexplained enrichment of about 0.8‰ , perhaps because this species secretes calcium carbonate further down in cooler waters. In most depth intervals for this core $\delta^{18}\text{O}_{\text{sac}}$ is greater than $\delta^{18}\text{O}_{\text{rub}}$, similar to cores from the Bay of Bengal and elsewhere^{9,8}. As the deduced surface conditions in which these two species secrete their shells do not differ significantly, this difference must be due to the reasons suggested above.

Complications from partial dissolution could also cause this behaviour⁹.

The figure shows this difference expressed as $\Delta^{18}\text{O}$ ($\delta^{18}\text{O}_{\text{sac}} - \delta^{18}\text{O}_{\text{rub}}$). The difference stays fairly constant at about 0.5‰ , except during two periods: a minimum around the LGM and a maximum around 12 kyr BP. The latter period also coincides with a major climate change in the Northern Indian Ocean and the Arabian Sea^{2,4-6,10,11}. The LGM was marked



The $\delta^{18}\text{O}$ difference between the planktonic forams *G. sacculifer* and *G. ruber* in the core SK-20-185 as a function of depth. Data from ref. 1.

by reduced upwelling in the Arabian Sea, resulting in the weakening of the southwest monsoon; higher sea-surface temperature (SST) in the Arabian Sea and higher salinities in the Bay of Bengal. The change of sign in Δ is also significant, although individual $\delta^{18}\text{O}$ values are more negative than the pre-LGM values, suggesting a reversal in the present-day temperature pattern. This is not surprising, because the SST pattern of the eastern Arabian Sea is influenced by the southwest monsoon: SSTs increase steadily from February, decrease from June and then reach another maximum in October. During a weak southwest monsoon at the LGM, the region would have behaved like middle and high latitudes, where SSTs increase steadily through the summer. The *G. ruber* isotope shift indicates a warming of the Arabian Sea surface by -2 °C , whereas the shift in $\delta^{18}\text{O}_{\text{sac}}$ suggests an increase of about 4 °C while its abundance peaks. This is consistent with previous studies which suggest that the Arabian Sea SST during the LGM was higher by about 2 °C , especially during August⁵.

The $\Delta^{18}\text{O}$ anomaly at 12 kyr before present is in the right sense $\delta^{18}\text{O}_{\text{sac}} > \delta^{18}\text{O}_{\text{rub}}$. This time period, the middle termination, was characterized by the return of a perhaps even stronger southwest monsoon². It is also recorded in the sediments from other Arabian Sea cores¹⁰. The isotope record in this core¹ shows very little change in $\delta^{18}\text{O}_{\text{sac}}$ but a negative excursion of about 0.5‰ in $\delta^{18}\text{O}_{\text{rub}}$. As $\delta^{18}\text{O}_{\text{rub}}$ is sup-

posed to reflect pre-monsoon surface conditions, it appears that during the middle termination, the pre-monsoon SSTs rose by about 2 °C . This may also have meant higher land temperatures, and, when coupled with the re-establishment of the Arabian Sea upwelling, a stronger southwest monsoon. The combined $\delta^{18}\text{O}_{\text{sac}}$ and $\delta^{18}\text{O}_{\text{rub}}$ records therefore suggest that, at the LGM the seasonal pattern of the Arabian Sea SST was reversed, whereas around 12 kyr before present it was much like the Holocene but with a larger amplitude.

R. V. KRISHNAMURTHY

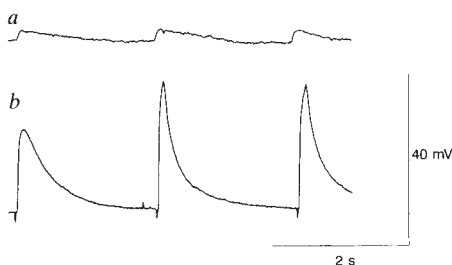
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1. Sarkar, A., Bhattacharya, S.K., Ramesh, R. & Rajagopalan, G. *Nature* **343**, 549–551 (1990).
2. Cullen, J.L. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **35**, 315–356 (1981).
3. Flood Control Series No. 11 (UN Economic Comm. for Asia and the Far East, New York, 1956).
4. Duplessy, J.C. *Nature* **295**, 494–498 (1982).
5. Prell, W.L. *et al. Quat. Res.* **14**, 309–336 (1981).
6. Duplessy, J.C., Be, A.W.H. & Blanc, P.L. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **33**, 9–46 (1981).
7. Wyrtki, K. *Oceanographic Atlas of the International Indian Ocean Expedition* (NSF, Washington, DC, 1971).
8. Deuser, W.G. in *Stable Isotopes in Earth Sciences* (ed. Robinson, B.W.) 55–60 (Dept of Scientific and Industrial Research, Wellington, New Zealand, 1978).
9. Berger, W.H. *Mar. Geol.* **11**, 325–358 (1971).
10. Borole, D.V., Kameswar Rao, K., Krishnamurthy, R.V. & Somayajulu, B.L.K. *Quat. Res.* **18**, 236–239 (1982).
11. Van campo, E., Duplessy, J.C. & Rossignol-Strick, M. *Nature* **296**, 56–59 (1982).

Role of nerves in hypertension

SIR—Although the cause of essential hypertension is unknown, our present knowledge suggests that sympathetic nerves have a key role in the development and/or maintenance of hypertension. The most compelling evidence for this has come from studies on the spontaneously hypertensive rat, an animal model in which a marked increase in blood pressure develops between one and three months of age. It has been shown that procedures used to prevent the development of sympathetic nerves can also prevent the development of hypertension^{1,2}. In investigating a functional role of the sympathetic nerves in this disorder by studies on mesenteric resistance vessels in this rat model, we have found evidence for markedly enhanced excitatory junction potentials in these nerves.

Sympathetic neural control of the small resistance arteries incorporates various mechanisms involving the primary transmitter noradrenaline and co-transmitters ATP and neuropeptide Y. Generally, the principal mode of constriction is activation of α -adrenoreceptors by noradrenaline causing constriction through release of calcium ions from intracellular stores via a



Excitatory junction potentials (EJPs) in the second-order ileal mesenteric arteries of a four-month-old hypertensive rat (b) compared to a four-month-old normotensive rat (a). The records show the largest EJPs recorded to supramaximal perivascular stimulation in the arteries studied (seven hypertensive and five normal rats; age range three to five months; stimulating pulse 100 V, 1 ms). The first EJP had a time course similar to that observed for EJPs in the other arteries from the hypertensives. However, the second and third EJPs in the train of stimuli had faster time courses. These two EJPs were facilitated becoming so large as to activate peaked, presumably voltage-dependent responses. The mean peak amplitude of the first EJP recorded for each artery from the same seven hypertensive and five normal rats was 14.4 ± 2.8 and 2.3 ± 0.7 mV, respectively. The vessels were studied *in vitro* in a standard physiological fluid at 35°C with recordings made using intracellular glass microelectrodes filled with 0.5 M KCl.

second messenger³. A second pathway for constriction is through activation of excitatory junction potentials which can summate to activate voltage-dependent calcium channels. Studies on the Wistar-Kyoto rat, the genetic control for the hypertensive rat, have shown that the resistance vessels have small excitatory junction potentials and undergo neurally mediated constriction predominantly through activation of α -adrenoreceptors⁴.

In hypertension, structural changes occur in the walls of the resistance vessels (see refs 2, 5 for reviews). There is a considerable proliferation of smooth muscle cells, leading to a thicker wall through increased numbers of these gap junction-coupled cells. The sympathetic innervation is also increased. But, as for the resistance arteries from control animals with normal blood pressure, this innervation remains in the adventitia, forming synapses only with the outer layer of smooth muscle. These hypertension-associated changes make traditional noradrenergic transmission increasingly ineffectual to the inner muscle. This arises through both the inner smooth muscle being further removed from the neural source of noradrenaline and there being

greater re-uptake of noradrenaline by the nerves.

The finding of markedly enhanced excitatory junction potentials in the hypertensive rat compared with the control (see figure) is therefore of considerable significance as it underlies a shift from chemical to electrical signalling. The enhanced sympathetic co-transmission would be expected to facilitate recruitment of all muscle layers, electrical signalling overcoming the limitations of the chemically mediated α -adrenergic mechanisms. Constriction should there-

fore exhibit an increased dependence on voltage-dependent calcium entry compared with the predominantly α -adrenergic mechanism of the normotensive control⁴. This might explain the success of calcium antagonists in combating hypertension in the hypertensive rat and could be true also in man.

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El Niño prediction

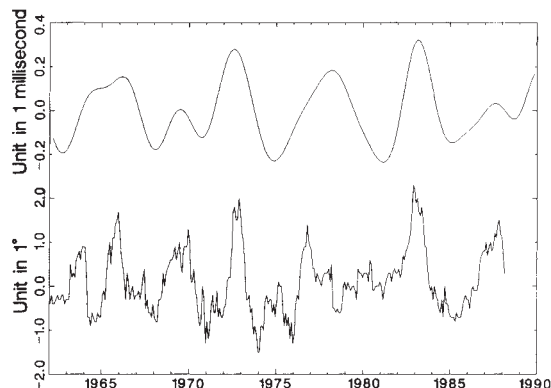
SIR—In November 1989 we made a forecast, based on analysis of changes in the length of day, that an El Niño event has been developing since the previous one in 1986–87 and that it would reach a peak intensity in the second half of 1990. We also predict that the sea-surface-temperature anomaly induced by the new El Niño event will be stronger than that in 1986–87.

Since the El Niño event in 1982–83, which was characterized by the anomalous warming up of the sea surface water in the equatorial area of the eastern Pacific, there has been significant progress in elucidating the relationship between the variation of the rate of the Earth's rotation and El Niño events^{1–7}. It has been suggested^{5,6} that El Niños could be predicted by changes in day length.

The variation of the Earth's rotation is influenced by many astronomical and geophysical factors^{8,9}. Applying the techniques of multi-stage filter (MSF) and leap-step autoregression (LSAR) that we have described^{10,11} could increase the reliability of predictions based on changes in day length.

Using astronomical data collected between January 1962 and October 1989 (refs 12–14) we obtained a series of changes in day length over a 30-day interval. According to the frequency band scales (2–7 yr) of seven El Niño events since 1962, the series of day-length changes can be processed with the band-pass filtering using MSF and LSAR (top trace in figure) and the monthly departures of the sea-surface temperature (SST) in the equatorial eastern Pacific from January 1962 to February 1988 (bottom trace of figure). The figure clearly shows that the interannual rate of the Earth's rotation decelerates when the sea water warms up and accelerates when the water cools down. Thus, every El Niño event usually occurs after the turn of the interannual rate of the Earth's rotation from acceleration to deceleration. We also find that the amplitudes

of the interannual change in day length are correlated with the strength of El Niño events. Since 1962, the two strongest El Niño events occurred in 1972 and in 1982–83, and in both the amplitudes of interannual variation in day-length change were larger. Thus the epoch and magnitude of El Niños can be predicted. Our analyses suggest that the late 1990



The interannual variation of day-length change from 1962 to 1989 (top) and the month departures of sea-surface temperature in the equatorial eastern Pacific area from 1962 to 1988 (bottom).

El Niño event will be stronger than that in 1986–87, and that it might last until 1991.

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1. Lee, R.M., Triggler, C.R., Cheung, D.W. & Coughlin, M.D. *Hypertension* **10**, 328–338 (1987).
2. Head, R.J. *Blood Vessels* **26**, 1–20 (1989).
3. Hashimoto, T., Hirata, M., Itoh, T., Kanmura, Y. & Kuriyama, H. *J. Physiol., Lond.* **370**, 605–618 (1986).
4. Angus, J.A., Broughton, A. & Mulvany, M.J. *J. Physiol. Lond.* **403**, 495–510 (1988).
5. Folkow, B. *Physiol. Rev.* **68**, 347–504 (1982).

1. Carter, W. E. *et al. Science* **224**, 957–961 (1984).
2. Rosen, R.D. *et al. Science* **225**, 411–414 (1984).
3. Chao, B.F. *Geophys. res. Lett.* **11**, 541–544 (1984).
4. Ren, Z.Q. & Zhang, S. G. *Kexue Tongbao* **6**, 444–447 (1985).
5. Eubanks, T. M. *et al. Earth Rotation: Solved and Unsolved Problems* 163–187 (Reidel, Dordrecht, 1986).
6. Zheng, D. W., Luo, S. F. & Song, G. X. *Science in China (Series B)* **32**, 729–736 (1989).
7. Song, G. X., Zheng, D.W. & Luo, S. F. *Acta astr. Sinica* **30**, 310–314 (1989).
8. Lambeck, K. *The Earth's Variable Rotation* (Cambridge University Press, New York, 1980).
9. Luo, S. F. *et al. Acta astr. Sinica* **15**, 79–84 (1974).
10. Zheng, D. W. & Dong, D. N. *Acta astr. Sinica* **27**, 368–375 (1986).
11. Zheng, D. W. *Progr. Astr.* **7**, 118–124 (1989).
12. Li, Z. X. in *Annual Report for 1984, D31-D36* (Bureau International de l'Heure, 1985).
13. *BIH Annual Report for 1982–1987* (Observatoire de Paris, 1983–88).
14. *IERS Annual Report for 1988 and Bulletin B for 1989* (Observatoire de Paris, 1989).

When are parasites clonal?

SIR—Keymer, May and Harvey drew attention in News and Views¹ to a paper by Tibayrenc and colleagues² who claimed that infections of most human parasitic protozoa are 'clonal', that is, derived from the reproduction of a few highly successful genotypes. This idea of clonality, with its implication that sexual reproduction is irrelevant in nature, is a stimulating one, and may be correct for some of these organisms. But we think it is wrong with respect to the most important agent of human malaria, *Plasmodium falciparum*.

First, sexual reproduction is an obligatory step in the parasite cycle in nature, and not a laboratory artefact. Apart from the rare cases of infection by blood transfusion, humans are infected with malaria by mosquito bites. The mosquitoes inoculate sporozoites, which are the haploid meiotic products of zygotes derived from mating of gametes in their stomach.

Second, genetic recombination has been directly demonstrated by infection of mosquitoes with two appropriately marked cloned lines, and by subsequent analysis of blood stage progeny^{3,4}. All the expected recombinants have been observed.

Third, there is extensive genetic diversity in *P. falciparum*. Creasey *et al.*⁵ studied 20 isolates from each of three countries, Thailand, Zimbabwe and Brazil, for 20 genetic loci determining isoenzymes, proteins detected by two-dimensional electrophoresis, antigens and drug-sensitivity, and found that every isolate had a unique combination of alleles at these loci.

Fourth, the blood of malaria-infected patients frequently contains two or more parasite genotypes⁶. When an individual mosquito takes in a mixture of clones, recombination between parasite genes is probably inevitable, and the enormous diversity of genotypes of *P. falciparum* seen in the wild is a consequence of this process.

Finally, Keymer *et al.*¹ and Tibayrenc *et al.*² drew attention to early work in our laboratory, in which we found that the genotypes GPI-1, ADA-1, LDH-1 and PEP-1 occurred in 10 out of 17 parasite samples⁷, implying that this is more than would be expected on a basis of free recombination between the loci for these enzymes. But the recurrent finding of the same genotypes in independent parasite

isolates can be used as evidence of a 'clonal' origin only if the respective allele frequencies in each locality and the number of loci are taken into account. ADA-1, LDH-1 and PEP-1 are by far the most common alleles of these enzymes in most African and South East Asian countries, from which these isolates originated. To say that parasites with this combination of alleles are clonal would be tantamount to saying that human beings who are blood group O and have haemoglobin A are 'clonal' (sexual reproduction in humans is not in dispute).

Thus the general model discussed by Keymer *et al.* cannot apply to *P. falciparum*. Infections of the South American parasite *Trypanosoma cruzi* may well exhibit clonal infection patterns⁸, but surely one group of parasitic protozoa cannot be generalized to others having very different types of reproductive cycle. In fact, genetic recombination in *P. falciparum* has important practical consequences, because parasites resistant to two different drugs can arise through this mechanism from singly resistant parasite clones. Thus, *P. falciparum* seems, unfortunately to our detriment, to reconcile the biological advantages of sexual reproduction in the mosquito with a devastatingly efficient aptitude for asexual reproduction in the liver and blood, which causes disease and death in the human host.

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SIR—We would like to offer a different perspective from that of Keymer *et al.*¹ on the recent paper by Tibayrenc and colleagues². With the exception of the malaria parasite *Plasmodium*, clonality is the conventional wisdom for parasitic protozoa, not a novel working hypothesis. Much of the recent work on recombination has focused on the African trypanosomes³ in which sexual reproduction clearly occurs, but is not obligatory and may not be common in nature. For *Leishmania*, *Entamoeba* and New World *Trypanosoma*, recombinants have been produced in laboratory experiments, but infrequently. Those who work with these genera have believed, and still believe, that reproduction can be assumed clonal until more substantial proof is offered to the contrary.

The assertion that the most important species of human malaria parasite is clonal deserves a closer look, for sexual reproduction in *P. falciparum* is obligatory.

However, Tibayrenc *et al.* tested for a significant deficit of recombinants by adopting the null hypothesis that *P. falciparum* exist in a globally panmictic population. In finding such a deficit among just 17 samples (from 15 isolates) from at least 8 African and Asian countries⁷, they may simply be demonstrating that parasites do not interbreed with sufficient frequency to counteract local selection, bottlenecks and drift. The lack of free recombination across continents is hardly surprising. Incidentally, Tibayrenc *et al.* listed as separate criteria for clonality over-represented multilocus genotypes and the absence of recombinants — surely two sides of the same coin. Another of their criteria is that correlations should exist between independent sets of genetic markers; in fact, such correlations seem hard to find within taxa^{10,11}.

Results of greater significance for *P. falciparum*, bearing on the management of drug resistance and the use of vaccines, will be obtained from more carefully formulated null hypotheses. Taking, say, an African village as a transmission unit, theory will need to take into account the frequency of mixed infections in individual people and mosquitoes, immunity, interactions between clones, linkage and crossing-over at meiosis. Carter and Voller¹² made a start some years ago, showing that enzyme genotype frequencies in two sets of isolates from The Gambia and Tanzania were consistent with independent assortment, showing no sign of the linkage disequilibrium expected in clonal lineages.

Although we do not offer here an ideal set of testable hypotheses, we suggest that future analyses of protozoan clonality, particularly of *Plasmodium*, will yield more convincing results if carried out with more data on restricted geographical and taxonomic scales — not by making intercontinental comparisons with just a handful of isolates.

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1. Keymer, A. E., May, R. M. & Harvey, P. H. *Nature* **346**, 109–110 (1990).
2. Tibayrenc, M., Kjelberg, F. & Ayala, F. *Proc. natn. Acad. Sci. U.S.A.* **87**, 2414–2418 (1990).
3. Walliker, D. *et al.* *Science* **236**, 1661–1666 (1987).
4. Fenton, B. & Walliker, D. *Genet. Res.* **55**, 81 (1990).
5. Creasey, A. M. *et al.* *Am. J. trop. Med. Hyg.* **42**, 403–413 (1990).
6. Thitthong, S. *et al.* *Trans. R. Soc. trop. Med. Hyg.* **78**, 242–245 (1984).
7. Sanderson, A., Walliker, D. & Molez, J.-F. *Trans. R. Soc. trop. Med. Hyg.* **75**, 263–267 (1981).
8. Tibayrenc, M. *et al.* *Trans. R. Soc. trop. Med. Hyg.* **78**, 519–525 (1984).
9. Tait, A. & Turner, C. M. R. *Parasit. Today* **6**, 70 (1990).
10. Angelici, M. C., Gramiccia, M. & Gradoni, L. *Parasitology* **99**, 301–309 (1989).
11. Pages, M. *et al.* *Molec. biochem. Parasitol.* **36**, 161–168 (1989).
12. Carter, R. & Voller, A. *Trans. R. Soc. trop. Med. Hyg.* **69**, 371–376 (1975).

Scientific Correspondence

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Science in the public eye

Dorothy Nelkin

Making Science Our Own. By Marcel C. LaFollette. University of Chicago Press: 1990. Pp. 260. Hbk. £35.95, \$51.75, pbk £14.75, \$20.75.

SCIENTISTS, these days, feel besieged. They face reduced public funding, accusations of fraud, and animal-rights protests. They compete with burgeoning social movements based on New Age philosophies and belief in holistic medicine that seem to reject the very essence of scientific thinking. The belief in progress that has long sustained large-scale support of science appears to be in decline, as new research projects confront public scepticism, if not explicit opposition.

Marcel LaFollette's *Making Science Our Own* is the latest in a number of books on science journalism that have recognized the growing tension in the relationships between science and society, and have explored the role of media images in explaining public attitudes. Arguing that the tensions between science and society reflect "images of science widely shared by American citizens throughout the century", LaFollette explores these shared images by examining the content of science articles in eleven popular US magazines from 1910 to the mid-1950s. She sees these magazines, accessible to millions of readers, as "representative of the content of all the mass media and as a measure of public sentiments".

In her engaging book, LaFollette reviews the images of science that seem to recur in the history of science popularization and reporting. They are highly stereotyped: science is an independent, unstoppable force; scientists are "male, white, brilliant, energetic, rational and dispassionate"; research inevitably leads to progress. But she also finds many contradictory images. Science appears in popular magazines as critically important to society, yet quite distant from public comprehension. New discoveries foster enormous expectations, yet they are also a source of fear and mistrust. Science writers convey an uneasiness with unchecked scientific growth, but also the sense that externally imposed restriction would have disastrous consequences. LaFollette describes how the popular magazines in the early twentieth century fuelled public expectations about the benefits of science with extravagant promises: cures for cancer, synthetic food to eliminate world starvation,

control over weather or unending sources of energy through nuclear power. But she also conveys their critical message that science could be destructive — a threat to religion and to fundamental human values.

Her documentation is often amusing and very familiar to contemporary media-watchers. LaFollette contrasts the media praise of "the men of science" (curious, dispassionate, burning the midnight

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Women in the laboratory — seen as "domestic, devoted to family and children" and the objects of condescension by the media.

oil) with the condescension towards the few women in the laboratories (domestic, devoted to family and children). Although she does not provide contemporary examples, such images persist. Just as a profile of Margaret Mead written in 1935 described how the anthropologist could make "corn fritters with crocodile eggs", so an article about Barbara McClintock written in 1983 has her "baking with black walnuts". Just as news articles in the 1930s created extravagant expectations about the promises of nuclear power, so the press in the 1980s promised wonders from cold fusion. Issues of secrecy, regulation and the high cost of science debated in the early part of the century still pre-occupy the press today. LaFollette's point about the importance of shared images would have been strengthened by more explicit comparisons with the content of current science reporting, for there are interesting and revealing continuities

that would buttress her argument.

The claim that public ambivalence towards science is rooted in the history of media images, however, rests on an important but not obvious assumption — that the media have a significant influence on public attitudes. LaFollette seems to accept the common response of scientists who tend to "blame the messenger" whenever there is criticism of science. Many scientists believe that the media are responsible for negative public attitudes towards science, that the tension between science and society reflects the poor public understanding of science, and that an adequately informed public would share the enthusiasm of scientists themselves. Thus, they try through public relations to convince journalists to project a more favourable public image. But this belief

Man Evans

oversimplifies the complexities of public attitudes towards science, and underestimates the importance of pre-existing attitudes in shaping readers' interpretation of media images. Also, for many magazine readers science articles are little more than a form of entertainment.

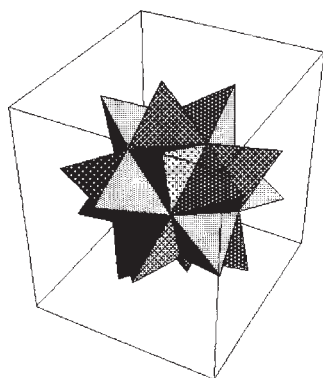
LaFollette appropriately observes that the scientific community has to some extent encouraged the tension between science and society through its own efforts to popularize and promote research by making extravagant claims. These efforts continue today and, indeed, have been formalized and extended with the expansion of public relations in science. The result is more promises, greater expectations and increased possibilities for disillusionment.

In her last chapter, the author describes both the changes and continuities in media coverage and in public attitudes towards science since 1955. But she stops short of analysing the nature of these changes, their sources in the changing social context of science and their significance. Nevertheless, this is an engaging and readable book that provides a wealth of material for those interested in the history of journalism and in the background of current public attitudes towards science. LaFollette concludes with the optimistic prediction that "a new generation of science communicators and journalists, alert to the moral, economic, and political implications of research, may succeed in conveying a realistic image . . .". But the material actually presented inspires this reader to say *plus ça change*. □

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Klein or Kipling?

Frances M. Brodsky

Immunology. By Jan Klein. Blackwell: 1990. Pp. 508. £45.

IN her foreword to Rudyard Kipling's *Just So Stories* (Weathervane, 1978), E. R. Choi states that "They fulfill the child's insatiable demand to know the how and why of everything. Kipling looks through the eyes of a child to view the world with naiveté and wonder." To my eyes, Jan Klein's new textbook *Immunology* is written from a similar point of view. True to the 'Just So' tradition, the first section of Klein's chapter on antibody structure and function is entitled "How immunoglobulins came to their name". Many other chapters begin in the same vein, with both a teleological as well as a historical introduction, giving the initial impression that *Immunology* is a highly personalized rationalization of why the immune system works the way it does. A closer look at the substance of the material presented in the body of each chapter, however, reveals a thoughtful attempt to organize and explain molecular and cellular immunology.

Klein's intent, as explained in his own preface, was to produce a textbook according to the definition provided in *Webster's New World Dictionary*: "a book giving instructions in the principles (or fundamental truths) of a subject of study". He aims to "find the fundamental truth in immunology and to separate it from hypotheses, regardless of how fashionable... It was my ambition to write a book of which a reader will say 20 years from now: 'There are no lies in it, but of course we now know much more...'." With this goal, Klein appears to have acknowledged Jonathan Howard's criticism (see *Nature* 326, 23-24; 1987) of his 1982 textbook, entitled *Immunology: The Science of Self-Nonself Discrimination* (Wiley, 1982) in which, according to Howard, "he (Klein) tells us not only everything he knew before he started work on the book, but also everything that he found out after he got going". Klein's new textbook takes the overdose of information presented in the earlier work, reorganizes it, updates it and distills it into a more coherent and logical exposition of the field. A test for me of whether Klein achieved his goal of dealing with "principles" was to look at his treatment of the fast-moving field of antigen presentation, in which "fashionable" hypotheses change on a monthly basis. Understandably, the section is already out of date, but there is little I could find that is fundamentally wrong with Klein's exposition of the principles involved and the problems that remain to be solved. A teacher assigning this textbook to students

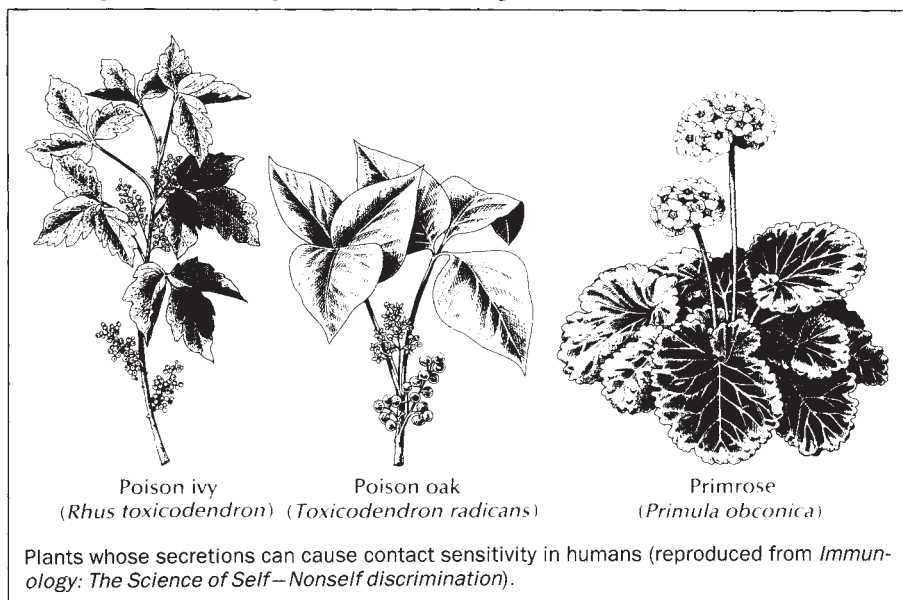
would be able to update the subject of antigen presentation in a lecture without having to contradict dogmatic statements made in the textbook. My impression of Klein's discussions of thymic education and tolerance is that these subjects could be dealt with similarly in a teaching situation. He has covered these controversial areas by presenting generalities rather than rehearsing arguments.

Klein's extremely personal writing style is an overwhelming characteristic of the textbook and has both good and bad aspects. He has organized the content of the book quite sensibly but clearly according to his own view of immunology. He first presents the known facts about cells and molecules involved in the immune system, then deals with molecular and cellular interactions that lead to an immune response, and finally discusses the regulation of the immune system and its malfunction in disease and allergy. Because of the anecdotal introductions to each chapter, Klein manages to present the biochemistry and genetics of immunologically active molecules in a context that avoids the cataloguing effect characteristic of some other textbooks, for example *Immunology* (Gower Medical Publishing, 1989) by Roitt, Brostoff and Male. At the same time, the molecular and genetic information provided in the individual chapters on immunoglobulins, T-cell receptors, lymphokines and their receptors, and complement is up to date and well organized. For example, the chapter on immunoglobulins contains an excellent summary of the known human and mouse Fc receptors. Unfortunately, the chapter on the major histocompatibility complex (MHC), although accurate in molecular details, suffers from an overdose of genetics and serology, Klein's research speciality.

Klein's detailed treatment of these aspects of the MHC is inappropriate considering the level at which most of the textbook is aimed but characteristic of his uneven use of explanatory digressions that vary from the sophisticated to the banal. Examples of the latter include explanations of X-ray crystallography and the reduction-alkylation of disulphide bonds in the chapter on immunoglobulins, and a long section describing the respiratory burst in macrophages which features a diagram of the molecular orbitals of oxygen to explain the formation of the superoxide anion. Such digressions are mainly confined to illustrations, so are not as distracting as they were when included in the text of Klein's previous textbook. In his comments on this earlier volume, Jonathan Howard questioned the value of illustrating the plants from which lectins are purified (which remain in the present text). He may still have a point, but it doesn't apply to all the pictures of plants in Klein's book (see figure). Based on

personal experience with poison ivy and poison oak, I can enthusiastically defend Klein's inclusion of pictures of plants that cause contact dermatitis. The cover illustration of Klein's new textbook shows liposomes, used as immunological adjuvants, and derives from another such ancillary explanatory illustration. This choice of cover is a little surprising, since Klein's opinion from Chapter 6 is that "In

wonder that the analogy did not extend to T-cell deletion and "Murder on the Orient Express", others may find this sort of story-telling condescending, unnecessary and even distracting to the point of complicating a relatively simple issue. But it certainly lightens up presentation of the potentially dense subject of immunology and, for me, made the book amusing to read.



the biology of our time, the two most stylish emblems are the DNA double helix and the 'Y' of the immunoglobulin molecule."

The reader of this textbook cannot fail to notice Klein's unusual and literary style. Although not as simplified as the explanations in Kipling's *Just So Stories*, analogies and parables abound. To cite a few examples: interleukin-1 is described as an "immunological Girl Friday," the domain structure of antibodies is compared to a "Lego" toy, and the interactions between the molecules on T cells with those of antigen-presenting cells are referred to as "group sex". The characteristic flavour of Klein's style is revealed in the following passage describing lymphocyte development: "... haemopoietic stem cells are like passengers in the sleeping compartment of the Orient Express. While things happen at a fast pace all around them, they rest peacefully, snuggled into the pillows of stromal cells. As the train jolts, jerks, and sways along the track, now and then one of (the) sleepers wakes up, relieves himself and then goes back to sleep again. For the stem cell, 'relieving itself' means mitotic division. . . These either return to the sleeping compartment (the stem cell pool) or are drawn into the whirlpool of activity outside the compartment . . . in the former case the stem cells have renewed themselves, whereas in the latter instance they have become committed to differentiation. . . ." While some readers may

Immunology targets a less sophisticated audience than Klein's last textbook and is easier to use. In *Immunology*, Klein is presenting "accepted truth" so he does not insert references in the body of the text but provides a general reference list at the end of each chapter. He gives historical background to explain the paradigms that preceded the immunological principles under discussion, but hardly gives any accounts of experimental work that contributed to the formulation of these paradigms. This level of treatment is certainly not suitable for a Ph.D. course, but could satisfactorily form the basis for an advanced undergraduate or medical school course in immunology, provided the lectures were coordinated with Klein's organization of the subject matter. In a course organized from a different perspective, both teachers and students might find the highly personal style and organization of the textbook a source of frustration. But *Immunology* provides a coherence to the subject, absent from those textbooks compiled by multiple contributing authors. Klein's impressive ability to produce large books single-handedly, and on a regular basis, itself deserves a 'Just So' story, "How Jan Klein writes his books". □

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Ecological discontinuity

Susan Harrison

Living in a Patchy Environment. Edited by Bryan Shorrocks & Ian R. Swingland. Oxford University Press: 1990. Pp.246. £35, \$75.

"SPATIAL patchiness and temporal variability can have important effects at every level of ecological organization, from the life histories and behavior of individuals, through the dynamics of populations, to the structure and function of communities of interacting plants and animals." May and Southwood thus set the tone, in their introductory chapter, for an edited volume which cuts across population biology's subdisciplines along the theme of patchiness.

The contents are primarily ecological, though evolution is not ignored. Topics include optimal foraging (McNamara and Houston), plant architecture (Sutherland), single-species population dynamics (Rosewell; Strong *et al.*; Gilpin), community structure (Dobson and Keymer; Hanski; Kitching and Beaver), conservation (Wright), group selection (Baker) and environmental sex determination (Swingland *et al.*). The chapters are brief (on average 20 pages), and each tends to review its authors' own work, although some authors provide general reviews and a few present new material.

Theory and empirical work are given roughly equal attention in the book as a whole. This is an admirable feat when one considers how far modelling has outrun experimentation in most of the subject areas, especially the spatial aspects of population dynamics and community structure. Some of the individual chapters integrate theory with data, such as Rosewell's on *Drosophila* (fruitfly) population dynamics and Hanski's on dung and carrion insects, demonstrating an approach with which a great deal of progress can be made.

Only a limited amount of continuity is possible between the chapters, given the range of issues addressed and of kinds and scales of 'patchiness' considered. Some themes recur among the chapters on population and community ecology. The role of random spatial aggregation in ameliorating competition, for example, is raised in a single-species model by Rosewell and in the context of competitive coexistence by Shorrocks and Dobson and Keymer. Hanski gives the idea its fullest development, describing random aggregation from its behavioural basis to its community-level consequences in dung and carrion insects. But, in general, the volume lacks unity. The only theme that

could have conceivably drawn together all the diverse contents is the contrast between a 'patchy' view of the phenomenon in question, and an older view of the same phenomenon in which spatial structure was ignored. Surprisingly though, not all the authors explicitly address the question of why spatial structure is important. (Or, why wholes are not just the sums of their patches.)

The book is a patchwork of interesting and in some cases excellent chapters, which nonetheless adds up to little more than the equivalent of several journal

articles by each of the contributors. It provides a convenient, if highly selective, introduction to current issues, but is far from a comprehensive work of reference. Population and community ecology seem ready for a bolder synthesis of recent work on spatial dynamics, better integrated if perhaps also more narrowly focused than this book. □

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Cache and carry

Hans Kruuk

Food Hoarding in Animals. By Stephen B. Vander Wall. *University of Chicago Press*: 1990. Pp. 445. Hbk \$87.50; £60.75, Pbk \$34.50; £23.95.

A LARGE majority of animal species are, in one way or other, food limited. Little surprise, then, that mechanisms have evolved for them to have some control over the availability of food resources to themselves or their kin. Most commonly, such control of access takes the form of territoriality, where feeding territories are defended against exploitation by conspecifics, enabling exploitation at optimal efficiency by territory 'owners'. An alternative, or complementary, method of controlling the vagaries of food availability is caching or storing at times of plenty. Widely practiced throughout the animal kingdom, food hoarding has important implications in ecology.

Even in its simplest form, hoarding is a highly complicated phenomenon;

watching it, one is assaulted by a range of questions about its exact mechanism, the neural processes involved, survival value in ecological terms, evolution, the problems involved in recovery of the caches, the effects of fruit hoarding on plant dispersion, and so on. Vander Wall in *Food Hoarding in Animals* deals with all of these, ably reviewing the evidence from about 1,500 references dealing with vertebrates, invertebrates, and with the plants of which the dispersal depends on animal food storing. The book is a *tour de force* which will be of great value to students of many disciplines.

Vander Wall himself has experimented on some spectacular examples of food caching in birds, among others in nutcrackers and jays. Much of his research, like that of others, centres on the recovery of caches, the spatial memory of the birds, and the kinds of environmental clues used. Despite ingenious experiments and fascinating laboratory results, one is still a long way from understanding how nutcrackers manage to recover such a large proportion of the seeds which they stored several months earlier, when storage took place under the colourful branches of

New in paperback

■ *The Ubiquity of Chaos* edited by Saul Klassner is published by the American Association for the Advancement of Science and draws together contributors in fields as disparate as physiology and quantum mechanics in which nonlinear dynamics act, price \$31.50.

■ *Cytokines* by Anthony Meager aims to provide a comprehensive and up-to-date introduction to the biology of this diverse group of secreted proteinaceous molecules. Published by Open University Press, price £15.99. (Hardback price £35.)

■ Cambridge University Press have just released the second edition of *Mass Spectroscopy* edited by H. E. Duckworth, R. C. Farber and the late V. S. Venkatasubramanian. The book is intended for students and for university and industrially based scientists and describes the principles of mass spectroscopy and its uses. Price £17.50, \$29.95.

■ In *Plasma Dynamics* published Oxford University Press, R. O. Dendy explains the fundamental concepts of plasma physics, price £12.50 (Also available in hardback, price £27.50.)

■ New from Gordon and Breach is *Reversing the Arms Race: How to Achieve and Verify Deep Reductions in the Nuclear Arsenal* edited by Frank von Hippel and Roald Sagdeev, price \$24. (Also available in hardback, price \$49.)

■ *Evolutionary Stability: Logical and Material Aspects of a Unified Theory of Biosocial Evolution* by Gebhard Geiger offers approaches for the solution of problems raised by the recent sociobiology debate. Published by Springer, price DM 79. □

trees in autumn, and recovery from under a metre of snow in winter.

Vander Wall discusses in detail the conditions under which caching could evolve, and goes some way to explain why some species do and others do not. The main argument is that some just do not have the raw material, "some genetically controlled trait from which natural selection could produce the advantageous characteristic". Dangerous ground, this; clearly, caching has evolved many times independently in different species, and over a huge period in evolutionary history. The fact that all canids cache food in exactly the same, unique manner suggests a very old trait in that family, whereas several quite different ways of hoarding food among hyaenids or felids indicate that there are many more recent arrivals of caching on the scene of evolution. It means that it is worthwhile looking for the selective advantages of not hoarding. This is what I would do if I had to explain why a crow beautifully caches his surplus chunk of carrion, whereas a herring gull in exactly the same conditions does not. This book inspires one to think this way; apart from providing an excellent review, it also stimulates by identifying many open questions. □

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REASONS

Saving it for later — the jay (*Garrulus glandarius*) caches surplus fruit and nuts. What makes some species cache whereas others do not?

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The GTPase superfamily: a conserved switch for diverse cell functions

Henry R. Bourne, David A. Sanders & Frank McCormick

Proteins that bind and hydrolyse GTP are being discovered at a rapidly increasing rate. Each of these many GTPases acts as a molecular switch whose 'on' and 'off' states are triggered by binding and hydrolysis of GTP. Conserved structure and mechanism in myriad versions of the switch—in bacteria, yeast, flies and vertebrates—suggest that all derive from a single primordial protein, repeatedly modified in the course of evolution to perform a dazzling variety of functions.

THE time is ripe for reviewing these GTPase proteins. It may never again be possible to capture them in a family portrait, because the family multiplies so rapidly and individual family members refuse to sit still. We now know several family members well enough, however, to recognize their cousins anywhere. Biochemical and molecular genetic analyses of newly discovered GTPases repeatedly demonstrate the importance of conserved primary structure and switching mechanism. Crystal structures of two of these proteins^{1–6} have recently revealed in three dimensions the structure of the switch and have identified its moving parts. These findings make the GTPases prime examples of evolution's wonderful ability to create functional diversity from conserved structure and mechanism.

This review will focus on the cellular functions of different families of GTPases, comparing different ways in which the basic cycle of GTP binding and hydrolysis has been harnessed to direct ribosomal protein synthesis^{7,8}, to mediate transmembrane signalling by hormones and light^{9–13}, to translocate nascent proteins into the endoplasmic reticulum^{14–17}, to control differentiation and cell proliferation^{18,19}, and to guide vesicular traffic within cells^{20–24}. The focus on cellular functions leaves no room for a comprehensive account of the relations between GTPase structures and molecular mechanisms, which are reviewed elsewhere^{25–28}.

At the outset a few 'housekeeping' comments are in order: We refer to members of the superfamily as 'GTPases' rather than 'GTP-binding proteins' or 'G proteins'. Although no term is perfect, the first evokes the crucial biochemical activity of these proteins more clearly than the other two, and the last term, 'G proteins', should be reserved for the signal-transducing heterotrimers. This review does not deal with several proteins known to consort with GTP, including tubulin, guanylyl cyclases, and GTP-utilizing kinases, which are only distantly related to the GTPase superfamily.

Cycle of GTP binding and hydrolysis

All GTPase switches go through the same cycle of reactions (Fig. 1). Binding and hydrolysis of GTP drive transitions among three conformational states: GDP-bound, 'empty', and GTP-bound. The 'empty' state serves as a (usually) transient intermediate in replacement of GDP by GTP in the guanine nucleotide binding site of the GTPase. The irreversibility of GTP hydrolysis makes the cycle unidirectional. The switching function of each individual GTPase in the cell is determined by the differing abilities of its conformational states to interact with specific macromolecules. For example, we describe the GTP-bound and GDP-bound forms of the signalling G proteins as 'active' and 'inactive', respectively, because the former activates effector enzymes, such as adenylyl cyclase, whereas the latter does not.

For most GTPases, the fraction of protein molecules in the 'on' position—that is, the GTP-bound form—depends on the relative rates of two reactions (Fig. 1): dissociation of GDP

from the GDP-bound form and hydrolysis of bound GTP. These rates are characterized, respectively, by two fractional rate constants, $k_{\text{diss-GDP}}$ and $k_{\text{cat-GTP}}$. The ratio between GTP-bound and GDP-bound forms is:

$$\frac{\text{GTPase} \cdot \text{GTP}}{\text{GTPase} \cdot \text{GDP}} = \frac{k_{\text{diss-GDP}}}{k_{\text{cat-GTP}}}$$

Note that this equation depends on a number of assumptions²⁹, most of which are valid most of the time—the concentration of GTP must not be limiting, for example, and GTP must bind rapidly to the empty site on a GTPase that has just released GDP.

The equation implies that the relative proportion of protein in the active GTP-bound state can be increased either by accelerating $k_{\text{diss-GDP}}$ or by reducing $k_{\text{cat-GTP}}$. For many GTPases, specific protein ligands regulate these rate constants. Those that increase $k_{\text{diss-GDP}}$ are called guanine nucleotide exchange proteins or release proteins (hereafter, GNRPs), whereas GTPase activating proteins (GAPs) increase $k_{\text{cat-GTP}}$.

Two well-characterized GTPases

The functional roles of different stages in the GTP-driven cycle are best understood for two GTPase families, the GTPases that direct ribosomal protein synthesis, exemplified by elongation factor Tu (EF-Tu), and the signal-transducing G proteins. Rather than repeat detailed reviews of these protein families^{7–11,13,30}, we shall focus on the versatility of the shared GTPase switch.

Elongation and initiation factors. Although features of primary structure are conserved among the elongation and initiation factors of ribosomal protein synthesis, from *Escherichia coli* to mammals, their GTPase cycles perform quite diverse functions, including (among others): formation of the 70S initiation complex, in which the bacterial initiation factor IF2 monitors the appropriate association of the 30S ribosomal subunit, messenger RNA, fMet-transfer RNA_f, other initiation factors, and, finally, the 50S subunit (which triggers GTP hydrolysis); kinetic proofreading, by EF-Tu, of the match between codon and anticodon; and translocation, mediated by EF-G, of peptidyl-tRNA from the A to the P site on the ribosome.

The GTPase cycle of EF-Tu (Fig. 2a) is representative. A guanine nucleotide release protein, EF-Ts, binds to the inactive form of EF-Tu and promotes dissociation of GDP; on subsequent binding of GTP, EF-Tu dissociates from EF-Ts. EF-Tu-GTP now can bind aminoacyl-tRNA, and the EF-Tu-GTP-aminoacyl-tRNA complex binds to the mRNA-programmed ribosome. Here, GTP hydrolysis, triggered by association with the ribosome, serves to monitor and enhance—by up to a hundredfold—the fidelity of translation.

This process, called kinetic proofreading, compares the duration of the codon-anticodon binding interaction to the times required for GTP hydrolysis and for the subsequent dissociation of EF-Tu-GDP^{8,31}. In the first proofreading step, an 'incorrect' (poorly matched) anticodon is more likely than a 'correct' anti-

TABLE 1 G Protein receptors and effectors

G Protein	Receptors for	Effectors	Signalling pathways
G _s	Adrenaline, noradrenaline, histamine, glucagon, ACTH, luteinizing hormone, follicle-stimulating hormone, thyroid-stimulating hormone, and others	Adenylyl cyclase Ca ²⁺ channels	↑ cAMP ↑ Ca ²⁺ influx
G _{olf}	Odorants	Adenylyl cyclase	↑ cAMP (olfaction)
G _{ti} (rods)	Photons	cGMP phosphodiesterase	↓ cGMP (vision)
G _{t2} (cones)	Photons	cGMP phosphodiesterase	↓ cGMP (colour vision)
G ₁₂ , G ₁₃ , G ₁₂ , G ₁₃	Noradrenaline, prostaglandins, opiates, angiotensin, many peptides	Adenylyl cyclase Phospholipase C Phospholipase A ₂ K ⁺ channels	↓ cAMP ↑ Inositol trisphosphate, diacylglycerol, Ca ²⁺ Arachidonate release Membrane polarization
G _o	Probably many, but not yet defined	Phospholipase C Ca ²⁺ channels	↑ Inositol trisphosphate, diacylglycerol, Ca ²⁺ ↓ Ca ²⁺ influx

Information summarized in this table is reviewed in greater detail in refs 9–11, 30 and 116.

codon to dissociate from the mRNA codon before GTP is hydrolysed; if it does so, the incorrect aminoacyl-tRNA will be rejected. If not, the second proofreading step ensues: The EF-Tu·GDP complex remains bound to its site on the ribosome for a brief period, thereby preventing transfer of the incoming aminoacyl-tRNA to the growing polypeptide chain. This transient association between the ribosome and the EF-Tu·GDP complex lasts long enough for a poorly matched anticodon to have a second change to dissociate from the codon, thus providing another opportunity for rejecting an incorrect aminoacyl-tRNA.

Note that GTP hydrolysis by EF-Tu is strictly conditional, that is, dependent on association of the EF-Tu·GTP·aminoacyl-tRNA complex with the ribosome, which serves as a GTPase activating protein, or GAP. The same is true of several other GTP-dependent elongation and initiation factors; in each case, the GTP-bound form of the factor promotes association between other macromolecules, and GTP is hydrolysed only after the appropriate association has been completed.

G proteins. Using a cycle (Fig. 2b) rather different from that of EF-Tu, α subunits of heterotrimeric G proteins transduce sensory and hormonal stimuli across the plasma membrane in eukaryotes. Cell-surface receptors, activated by extracellular ligands or—in retinal rod and cone cells—by photons, act as GNPs by promoting dissociation of GDP from G proteins, which are attached to the cytoplasmic leaflet of the plasma membrane. Entry of GTP into the guanine nucleotide binding site of the α subunit triggers dissociation of the $\alpha\beta\gamma$ complex from the receptor and separation of α -GTP from the $\beta\gamma$ complex (Fig. 2b). α -GTP then activates an effector enzyme or ion channel. Hydrolysis of bound GTP by an intrinsic GTPase activity of the α chain terminates its interaction with the effector, and α -GDP binds to $\beta\gamma$; the resulting $\alpha\beta\gamma$ -GDP complex is available for activation by another receptor molecule.

At present, eight different mammalian G proteins can be biochemically defined and associated with transduction of specific signals (Table 1). They are distinguished primarily by structural and functional differences among α subunits; the different β and γ chains so far identified seem for the most part to be functionally equivalent^{9–11,30}. The family is growing rapidly: molecular cloning has recently identified complementary DNAs encoding five additional α chains^{32–34}, whose cellular functions are not yet known.

Compared with other protein signalling machines^{35,36}, the G proteins stand out as virtuosos at sorting and amplifying transmembrane signals. Each G protein determines the flow of information from a specific subset of receptors to a similarly specific and usually smaller subset of effector molecules. The first specific step in sorting signals occurs in activation of guanine nucleotide exchange (Fig. 2b): The G protein selectively interacts with the correct activated receptor (or class of receptors), ignoring the

numerous structurally similar but functionally inappropriate receptors also present in the plasma membrane.

In the second specific step, the GTP-bound active form of the α chain binds to and activates the correct effector protein—for example, adenylyl cyclase for G_s, cGMP phosphodiesterase for retinal transducin (G_t), and phospholipases or K⁺ channels for other G proteins (Table 1). The α chains of G proteins—denoted α_s for G_s, α_t for G_t, and so on—contain the structural determinants responsible for correct sorting of signals transmitted from receptors to effectors; a $\beta\gamma$ complex is required for receptors to interact with α and increase its $k_{\text{diss-GDP}}$, but is thought not to participate directly in discriminating among different subsets of receptors^{9–12,30}. The effector sorting function played by α_s (and other α chains) is simpler than the parallel function of EF-Tu. To promote cAMP synthesis, α_s need only bind to a single effector molecule, adenylyl cyclase, whereas peptide elongation requires that EF-Tu bind both to an aminoacyl-tRNA and to a programmed ribosome in order to promote their productive association (compare panels *a* and *b* of Fig. 2).

G proteins use GTP hydrolysis as a timer for amplifying signals. In retinal rod cells, for example, each photorhodopsin generates several hundred GTP-bound α_t molecules in less than 0.5 s; each active α_t persists in the active state long enough to find and activate a cGMP phosphodiesterase molecule, thereby triggering a rapid decrease in cGMP concentration³⁷. In the adenylyl cyclase system, signal amplification differs in detail but not in underlying mechanism. Each hormone-receptor complex is short-lived, persisting for less than 1 s, but generates one or more GTP-bound α_s chains that remain active for 10 s or more^{10,38}. In both cases amplification depends on the ability of

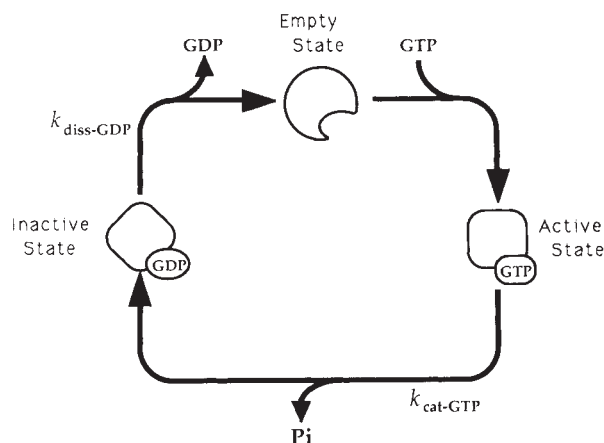


FIG. 1 The basic GTPase cycle. See text for details.

the bound GTP to make the α chain 'remember' its activation after it has dissociated from the receptor.

Although both EF-Tu and the G proteins use GTP hydrolysis as a timing device, the different requirements of kinetic proofreading and signal amplification dictate radically different mechanisms for controlling GTPase activity. In the first case, the kinetic proofreading process requires that association with the ribosome accelerate, by a factor of $>10^5$, the vanishingly low intrinsic $k_{\text{cat-GTP}}$ of EF-Tu^{7,31}. The rate at which the timer runs (that is, the ribosome-dependent $k_{\text{cat-GTP}}$) must be precisely engineered in order to optimize both the fidelity and efficiency of translation⁸. Even more important, the proofreading timer must begin its countdown at exactly the right moment—that is, when the EF-Tu·GTP-aminoacyl-tRNA complex binds to the programmed ribosome. This is why GTP hydrolysis by EF-Tu must be so strictly conditional.

Signal amplification by the G protein does not impose such stringent requirements. The G proteins seem to amplify signals by using a fixed $k_{\text{cat-GTP}}$ that is built into the structure of G protein α chains. Compared with the ribosome-triggered GTPase of EF-Tu, the G proteins hydrolyse GTP much more slowly^{12,39,40} ($k_{\text{cat-GTP}} \sim 2\text{--}4\text{ min}^{-1}$). This slow rate of inactivation makes it possible for the G protein timer to begin its countdown when α -GTP dissociates from receptor and $\beta\gamma$, rather than having to wait for α -GTP to bind the effector. No extrinsic activators (GAPs) of α chain GTPase—analogueous to the ribosome in the case of EF-Tu—have been identified. Apparently, the intrinsic, unvarying rate of GTP hydrolysis engineered into the structure of the α chain is slow enough to amplify signals and rapid enough to allow physiologically appropriate turn-off of signal transmission.

In the superfamily of GTPases, failure to make use of an extrinsic GAP makes G protein α chains exceptional; GTP hydrolysis in all other well-studied family members is conditional.

Pheromone signalling in yeast. In *Saccharomyces cerevisiae*, mating pheromones bind to receptors that activate a G protein composed of α and $\beta\gamma$ subunits structurally similar to the corresponding polypeptides of vertebrates^{41–44}. This yeast G protein adds a significant new wrinkle to our understanding of the adaptability of the GTPase cycle. Just as observed with mammalian G proteins, the ligand-activated pheromone receptor promotes binding of GTP to the G protein α chain, causing it to dissociate from $\beta\gamma$. Genetic evidence⁴⁴ makes it clear that $\beta\gamma$, rather than α , carries the signal to the pheromone effector pathway. Genetic deletion of the yeast α chain causes constitutive activation of the pheromone response pathway. This contrasts sharply with the abrogation of signalling—that is, of hormonal stimulation of adenylyl cyclase—produced in a cultured animal cell by genetic loss of α_s ^{45–47}.

Rather than activating a signalling pathway directly, the yeast α chain seems to act primarily as a negative regulator (in its GDP-bound state) that binds and inactivates $\beta\gamma$. The GTP-bound form of α chain, however, probably serves not only to release $\beta\gamma$ but also to produce a distinct effect on its own. Genetic evidence⁴⁸ strongly suggests that α -GTP in this system triggers a secondary pathway that opposes and can eventually overcome part of the differentiation programme mediated by $\beta\gamma$.

These findings show that $\beta\gamma$ can directly transmit signals, at least in *S. cerevisiae*. In vertebrate signalling systems the $\beta\gamma$ complex clearly performs other functions, including presenting the α chain to activated receptors. Several pieces of evidence, summarized elsewhere⁴⁹, suggest that $\beta\gamma$ may act as a direct transmitter of signals in multicellular eukaryotes as well.

Ras proteins

Ras proteins, ubiquitous in eukaryotes, have become objects of especially intensive study because mutations in mammalian *ras* genes cause neoplastic transformation. The 21K protein products of these genes—referred to hereafter as p21^{ras}—comprise

by far the best characterized subgroup of proteins in the ever-expanding tribe of small (20–35K) GTPases (see Table 2 and discussion, below). The molecular behaviour and three-dimensional structure of p21^{ras} furnish a marvellous framework for analysing relations of structure and function^{25–28}. We remain profoundly ignorant, however, of answers to many key questions regarding control of cell proliferation and differentiation by p21^{ras}. The Ras proteins of *S. cerevisiae* provide suggestive analogies and an additional set of beguiling puzzles.

Mammalian p21^{ras}. The three *ras* genes of mammals¹⁸, *H-ras*, *K-ras*, and *N-ras*, encode 21K proteins that control regulatory pathways critical for normal proliferation and differentiation. The p21^{ras} protein promotes neoplastic transformation of fibroblasts¹⁸, maturation of *Xenopus* oocytes⁵⁰, and differentiation of PC12 cells^{51,52}. In each case, it seems that the GTP-bound form of p21^{ras} is responsible for the effect. However, the biochemical pathways upstream or downstream have yet to be identified (see Fig. 3a).

In vitro, pure p21^{ras} exhibits quite slow rates of GDP dissociation and GTP hydrolysis (~ 0.008 and 0.02 min^{-1} , respectively)^{53,54}, implying the existence of GNRPs that catalyse guanine nucleotide exchange and of proteins that stimulate GTPase. Indeed, three groups have identified GNRPs for p21^{ras} (refs 55–57), and a GAP for p21^{ras} has been identified²⁹ and structurally characterized by molecular cloning^{58,59}.

Oncogenic mutations in p21^{ras} clearly demonstrate the physiological importance of GTPase stimulation by GAP. One class

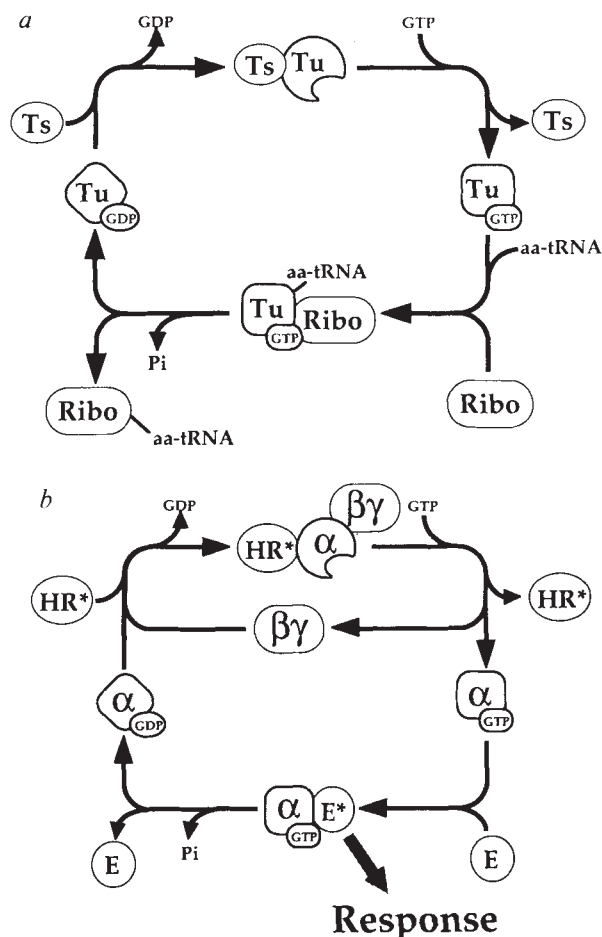


FIG. 2 GTPase cycles of EF-Tu (a) and α subunits of vertebrate G proteins (b). Tu, Bacterial EF-Tu; Ts, bacterial EF-Ts, the exchange protein for EF-Tu; aa-tRNA, aminoacyl-tRNA; Ribo, mRNA-programmed ribosome; α , G protein α subunit; $\beta\gamma$, G protein $\beta\gamma$ complex; HR*, hormone-receptor complex (which catalyses guanine nucleotide exchange); E and E*, inactive and active effector proteins (targets of G protein regulation), respectively.

of these mutations, often found in human tumours, locks the protein into its active, GTP-bound state. These mutations allow p21^{ras} to bind GAP but prevent GAP from increasing $k_{\text{cat-GTP}}$ ^{19,58}. Sensitivity to this effect of GAP keeps normal p21^{ras} mostly in the inactive GDP-bound form, whereas the GAP-resistant mutant proteins accumulate in the GTP-bound active form^{29,52,60}. A second class of oncogenic mutant Ras proteins⁶¹⁻⁶³ exhibits a greatly increased $k_{\text{diss-GDP}}$, which presumably allows rapid GTP-induced activation. Although mutant Ras proteins of this class have not yet been found in human tumours, their ability to trigger neoplastic transformation in cultured cells underscores the key role of GDP dissociation as a limiting step in the normal GTP-driven cycle of p21^{ras}.

The physiological roles of GAP are not well understood. Compelling evidence indicates that GAP acts as a negative regulator of p21^{ras}, inactivating the protein by inducing it to hydrolyse GTP; other evidence suggests that GAP may also act as a positive effector of p21^{ras} (Fig. 3a).

Many observations illustrate negative regulation by GAP. For example, human GAP activates the GTPase of p21^{ras} and decreases its stimulatory effect on yeast adenyl cyclase when both proteins are expressed in *S. cerevisiae*^{60,64}. In fibroblasts, overexpression of GAP suppresses neoplastic transformation⁶⁵ induced by p21^{c-ras}. Recent observations⁶⁶ point to GAP as the principal regulator of the fraction of p21^{ras} in the GTP-bound form in intact T lymphocytes: activation of the T-cell receptor markedly increases p21^{ras}-GTP; activation of protein kinase C produces the same effect and simultaneously inactivates GAP's ability to trigger GTP hydrolysis by p21^{ras}.

The idea that GAP may also serve as a positive effector of p21^{ras} action (Fig. 3a) is supported by indirect evidence, including mutations in a small region of the Ras protein that prevent neoplastic transformation and simultaneously prevent the protein from interacting with GAP (summarized in refs 19, 67-69). More direct evidence in favour of an effector role for GAP

comes from recent experiments⁷⁰, in which recombinant GTP-bound p21^{ras} prevents coupling of a G protein to the M₂ muscarinic acetylcholine receptor in patches of cardiac plasma membrane, and anti-GAP antibodies prevent this effect. Recombinant GAP also prevents coupling, and this effect too is blocked by an anti-p21^{ras} antibody known to block interaction between GAP and Ras proteins. These results indicate that the uncoupling effect results from concerted action of p21^{ras}-GTP and GAP. GAP clearly is necessary for p21^{ras} to act in this system, supporting the strong inference that GAP performs an effector function distinct from its effects on GTP hydrolysis.

In summary, GAP clearly acts as a negative regulator and probably also serves, in some systems at least, as a positive effector of p21^{ras} (ref. 69). It is unlikely that GAP is the sole effector of p21^{ras} in these systems, however⁶⁵.

Ras proteins of *S. cerevisiae*. Extensive genetic analysis indicates that the protein products of the *RAS1* and *RAS2* genes stimulate adenyl cyclase in *S. cerevisiae*. Addition of purified Ras proteins to membrane extracts prepared from *ras1⁻ras2⁻* cells activates cAMP synthesis in a GTP-dependent fashion^{71,72} (for review see ref. 68). It is not clear, however, whether the Ras proteins stimulate adenyl cyclase directly or through as yet unidentified protein intermediates (Fig. 3b). Genetic evidence indicates that the Ras proteins of *S. cerevisiae* regulate at least one pathway, not yet biochemically identified, in addition to adenyl cyclase^{73,74}.

Genetic evidence⁷⁵⁻⁷⁸ strongly suggests that the protein product of the *CDC25* gene serves as a catalyst of guanine nucleotide exchange for the Ras1 and Ras2 proteins, as depicted in Fig. 3b; presumably this is why Cdc25 is necessary for maintaining active cAMP synthesis, except in yeast cells carrying Ras proteins with decreased GTPase activity. The physiological role of cAMP in *S. cerevisiae* suggests that the exchange function of Cdc25 should be modulated by nutrients and metabolites that regulate progression through a cAMP-requiring stage in the yeast cycle. No such regulatory ligands have yet been identified, however.

GNRPs for most other GTPases—including p21^{ras} and the other 20-35K proteins—are just beginning to be identified, and their regulatory roles remain poorly defined. A C-terminal fragment of Scd25, a yeast protein structurally related to Cdc25, can catalyse guanine nucleotide exchange on RAS proteins⁷⁸, suggesting that GNRPs for p21^{ras} and other small GTPases may also turn out to be catalysed by Cdc25-like proteins.

Yeast Ras proteins seem to hydrolyse GTP conditionally, like p21^{ras} and EF-Tu, but unlike G protein α chains. That is, yeast Ras GTPase activities depend on the presence of other proteins, probably those encoded by the *IRA* genes⁶⁰ (Fig. 3b). Both in function and in the sequences of significant stretches of amino-acids, the Ira1 protein resembles the vertebrate GAP, which stimulates GTP hydrolysis by p21^{ras} (see below).

Questions. There are several unsolved puzzles. First, there is the glaring absence of clearly identified biochemical pathways that mediate regulation of cell proliferation and differentiation by p21^{ras}. In addition to its ability to stimulate $k_{\text{cat-GTP}}$, GAP can apparently mediate an effector function, but how it does so is unknown. One possibility is that GAP is (or stimulates) an enzyme whose metabolic products are responsible for effects of p21^{ras}. Although tantalizing, recent reports that GAP associates with and serves as a substrate for phosphorylation by protein tyrosine kinases⁷⁹⁻⁸² do not tell us how (or even whether) p21^{ras} may be involved in the GAP-kinase interaction. Does phosphorylation of GAP on tyrosine residues change its affinity for p21^{ras}-GTP or alter its GTPase stimulating activity? Does a kinase-GAP-p21^{ras}-GTP complex bind to and regulate some downstream effector molecule?

Furthermore, unlike the case of EF-Tu, we have no plausible rationale for the conditional GTPase activity of either mammalian or yeast Ras proteins. In the mammalian case, a single GAP protein can both stimulate GTP hydrolysis and may—at

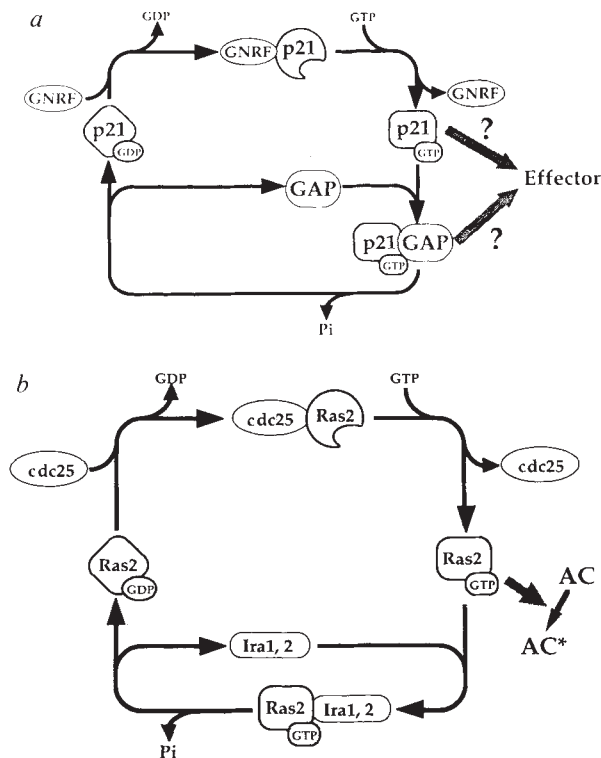


FIG. 3 GTPase cycles of p21^{ras} (a) and Ras2 of *S. cerevisiae* (b). GNRP, Guanine nucleotide release protein for p21^{ras}; GAP, GTPase activating protein; Cdc25, yeast protein thought to catalyse exchange of GTP for GDP on Ras proteins; Ira1,2, yeast proteins that stimulate GTPase activity of Ras proteins; AC, adenyl cyclase.

TABLE 2 Small (20–35K) GTPases

Subclass*	Approximate number		Representative proteins	Cellular function
	Mammals	Yeast		
Ras and Ras-like	6	3	Ha-, Ki-, N-ras (mammals) R-ras, Rap proteins (mammals) Ras1, Ras2 (<i>S. cerevisiae</i>)	See text See text See text
Ypt1/Sec4	8	2	Rab3 (mammals) Ypt1 (<i>S. cerevisiae</i>) Sec4 (<i>S. cerevisiae</i>)	Localized in synaptic vesicles ^{117–120} Transport, ER to Golgi (see text) Vesicle transport to plasma membrane (see text)
Rho	3	3	Rho C (mammals) CDC42 (<i>S. cerevisiae</i>)	Exoenzyme C3 of <i>C. botulinum</i> ADP-ribosylates rho protein and disrupts actin microfilaments ^{121–124} Inactivating mutation disrupts cytoskeleton and cell polarity ^{125,126}
ARF-like	1	3	ADP-ribosylation factor (ARF)	Required for modification of α_s catalysed by cholera toxin ^{127,128}

* The subclasses of small GTPases are largely defined by similarities of primary structure in regions known to participate in formation of the guanine nucleotide binding pocket. See ref. 129 for a more detailed analysis.

least for some actions of $p21^{ras}$ —also contribute directly to effector regulation. In contrast, GTPase activation of Ras proteins by Ira1 and Ira2 in *S. cerevisiae* seems to occur without any interaction between the Ira proteins and one well-established effector, adenylyl cyclase; the Ira proteins might, however, interact with other (not yet identified) effector proteins.

Finally, no ligand or regulatory pathway—analogueous to the hormone receptors coupled to G proteins—has been found to promote exchange of GTP for GDP bound to $p21^{ras}$ or to yeast Ras proteins. Indeed, provocative recent observations⁵⁶ strongly suggest that guanine nucleotide exchange may be catalysed by an unregulated, constitutively active GNP analogues to EF-Ts. In permeabilized T lymphocytes, radioactively labelled GTP bound to $p21^{ras}$ at a rapid rate, consistent with an effective $k_{diss-GDP}$ of $\sim 1 \text{ min}^{-1}$. This rapid rate—many-fold faster than the intrinsic $k_{diss-GDP}$ of $p21^{ras}$ would allow—was observed in unstimulated cells. Activators of protein kinase C did not affect the rate of guanine nucleotide exchange on $p21^{ras}$, but did increase the fraction of $p21^{ras}$ in the GTP-bound form, apparently by decreasing $k_{cat-GTP}$ (by inactivation of GAP) rather than by increasing $k_{diss-GDP}$. Unpublished experiments (J. Downward, personal communication) suggest that the rate of guanine nucleotide exchange is similarly rapid in unstimulated permeabilized fibroblasts as well as lymphocytes.

These findings call into question the prevailing view that Ras proteins are signal transducers analogous to the heterotrimeric G proteins. Certainly, the role of yeast Ras proteins as necessary cofactors for cAMP synthesis makes them prime candidates for roles in signal transduction, although no first messengers have been identified. Alternatively, the combination of conditional GTPase activity and rapid, constitutive exchange of GTP for GDP suggest that the Ras GTPase cycle resembles that of EF-Tu more than that of G protein α chains, and that the G protein analogy may have misled us. Is it possible that Ras uses the energy of GTP not to transduce regulatory signals but rather to monitor or promote proper assembly of macromolecular complexes in membranes? To be sure, it is hard to imagine what these complexes might be—but not, perhaps, more difficult than finding the elusive and perhaps equally imaginary signalling pathway mediated by $p21^{ras}$.

The NF1 gene

Patients who inherit an inactivated mutant version of the *NF1* gene suffer from neurofibromatosis type 1 (NF1), a propensity to form multiple tumours that emerge from peripheral nerves. The large (>2,500 amino acids) protein product of the *NF1* gene includes a 360-residue region similar in sequence to the catalytic portion of Ras-GAP; a much larger stretch of $\sim 1,500$ residues, including the same 360-amino-acid sequence, strikingly resembles the yeast *IRA1* product⁸³. Already several laboratories

are exploiting the exciting possibility that the neurofibromas result from failure of the NF1 protein to stimulate GTPase activity of $p21^{ras}$ or a Ras-like protein.

The long stretch of sequence similarity between the NF1 and Ira1 proteins, greatly exceeding the size needed to promote GTP hydrolysis, is especially intriguing. Inactivating a Ras-like protein is probably only one of the functions shared by these two proteins; genetic experiments in *S. cerevisiae* may reveal other shared functions.

Other small (20–35K) GTPases

This heterogeneous collection of small GTPases already comprises at least 18 distinct proteins in vertebrates and eight in *S. cerevisiae*, and the number is growing rapidly. Many of these proteins were discovered (often by several laboratories) because they bind GTP or because their cDNAs hybridize with nucleotide sequences derived from *ras* genes. Consequently, the plethora of different names for individual small GTPases is accompanied by a dearth of information about their cellular functions, so that these proteins are most reasonably classified according to similarities of primary structure (Table 2).

Genetic and other evidence hints that individual small GTPases are involved in regulating the cytoskeleton and/or intracellular transport of vesicles (Table 2). Whether structural similarities within subcategories of small GTPases reflect conserved sets of cellular functions is now known. More generally, precedents provided by G proteins, EF-G, and EF-Tu suggest that individual small GTPases will interact with GNRP and GAPs to transduce signals, translocate macromolecules, or monitor specific assembly of macromolecules. Aside from the Ras proteins, only one small GTPase—the Sec4 protein of *S. cerevisiae*—has been studied well enough even to provide a framework for exploring its function at a molecular level, as described below.

Guanine nucleotide exchange and regulation of GTPase. Biochemical analysis of most of the recombinant small GTPases synthesized in *E. coli* has revealed very low $k_{cat-GTP}$ and $k_{diss-GDP}$ values, suggesting that their cellular functions must involve interactions with proteins that regulate GTP hydrolysis and dissociation of GDP. These interactions are likely to be specific, at least with respect to subgroups of small GTPases.

For example, the GAP protein previously found²⁹ to promote GTPase activity of $p21^{ras}$ turned out to stimulate the GTPase activity of a Ras-related protein, R-ras, but did not stimulate GTP hydrolysis by a mammalian rho protein; a second GAP activity, resolved from Ras-GAP, was found to stimulate GTP hydrolysis by rho, but not by $p21^{ras}$ or R-ras protein⁸⁴.

Another example may hint at a specific cellular function for a GTPase–GAP interaction: Ras-GAP interacts with Rap1 proteins, but this reaction does not stimulate Rap1 GTPase activity.

TABLE 3 Evidence that SEC4 protein mediates vectorial transport of vesicles

Experimental observation	Interpretation
Sec4 protein distributed in late secretory vesicles, plasma membrane, and cytoplasm ¹⁰⁸	Sec4 may cycle among these compartments
Mutational inactivation of Sec6, which causes accumulation of late secretory vesicles, causes decrease in Sec4 in plasma membrane, increase of Sec4 in late secretory vesicles ¹⁰⁸	Suggests Sec4 normally moves from vesicle fraction to plasma membrane in concert with vesicle transport, then returns via cytoplasm to secretory vesicles
Sec4 mutation in guanine nucleotide binding pocket, which presumably decreases affinity for binding guanine nucleotides, inhibits secretion and causes accumulation of late secretory vesicles (even in presence of normal Sec4 ⁹⁷)	Sec4 protein with 'empty' guanine nucleotide binding site cannot 'carry' vesicle to plasma membrane; a bound guanine nucleotide (presumably GTP) is required for this step

Instead, a relatively stable Rap1·GAP complex is formed⁸⁵. This enables Rap1 (in its GTP-bound form) to act as a competitive inhibitor of GAP-stimulated GTPase of p21^{ras}, at least *in vitro*^{85,86}. The GTPase activity of Rap1 is controlled by a different GAP, which is unable to affect the GTPase activity of Ras proteins⁸⁷. The ability of Rap1 to antagonize neoplastic transformation⁸⁸ by p21^{ras} could be related to its ability to compete with p21^{ras} for interaction with GAP. Indeed, the affinity of Rap1 for GAP is about 100 times higher than that of p21^{ras} for GAP⁸⁵.

Some of the GNRPs for small GTPases will probably show homology to the CDC25 gene product of *S. cerevisiae*, described above. Proteins with precisely the opposite effect, such as the recently discovered GDP-dissociation inhibitors (GDI), act on two different small GTPases^{89,90}. A GDI from bovine brain shows some sequence similarity to Cdc25⁹¹. The observation that this protein can prevent binding of the GDP-bound form of a small GTPase to synaptic vesicles and membranes⁹² hints at a possible physiological role for this GDI. It seems likely that GDI proteins specific for other small GTPases will soon be discovered.

Modification of C-terminal cysteines. All the small GTPases contain at least one cysteine residue near the C-terminus. In the Ras, Ras-related, and rho proteins this cysteine forms part of a characteristic tetrapeptide motif, C-A-A-X (A representing an aliphatic residue and X any residue), at the extreme C terminus. Genetic and biochemical studies⁹³⁻⁹⁵ have shown that the A-A-X tripeptide of p21^{ras} is removed soon after translation, and that the cysteine is carboxymethylated and polyisoprenylated. This complex series of processing steps is required for attaching p21^{ras} to the plasma membrane and for its biological function^{67,96}.

Many other small GTPases, including the Sec4 and Ypt1 proteins of *S. cerevisiae* (Table 2), contain a pair of C-terminal cysteines, at least one of which is essential for proper membrane localization and function^{97,98} and probably serves as a site for carboxymethylation and isoprenylation^{99,100}.

Vesicle transport and secretion. GTP and GTP analogues profoundly alter rates of vesicular transport in experimental models of endocytosis and exocytosis^{24,101-105}. Many of these effects are probably mediated by small GTPases functionally analogous to two proteins of *S. cerevisiae* that are thought to direct vesicular traffic at different stages of the exocytic pathway. Inactivating mutations of the YPT1 gene^{21,23,106} interrupt the yeast exocytic pathway at an early stage, at or soon after transport between the endoplasmic reticulum (ER) and Golgi apparatus. Proteins

immunologically related to the Ypt1 protein are found associated with the Golgi apparatus of mammalian cells²³, and a mouse-derived Ypt1-like protein can replace Ypt1 function in *S. cerevisiae*¹⁰⁷. Mutations that inactivate the Sec4 protein interfere at a later stage of the yeast secretory pathway, preventing mature secretory vesicles from interacting with the plasma membrane.

Genetic and biochemical studies of Sec4^{20,97,108,109} are beginning to show how the GTPase cycle may be adapted to meet a critical requirement of vesicular transport in eukaryotic cells: Every vesicle that buds off a donor membrane compartment (ER, Golgi, endosome, plasma membrane) must be targeted specifically to the appropriate acceptor membrane compartment. It seems likely that each targeting function is mediated by one of a conserved set of protein machines designed to promote and/or monitor vectorial transport of vesicles. The idea that individual GTPases have specific roles in such targeting machines receives support from the recent observation that each of three members of the Ypt1/Sec4 subfamily is localized to a specific exocytic or endocytic subcompartment in a mammalian cell line¹¹⁰.

The Sec4 protein is emerging as a paradigm for one component of such a protein machine. Figure 4 depicts the GTPase cycle proposed for Sec4^{24,97}, in which the protein moves from cytoplasm to mature secretory vesicle to plasma membrane and finally returns to the cytoplasm. The function proposed for Sec4 and other GTPases in directing vesicle transport closely resembles that of EF-Tu in ribosomal synthesis (compare Figs 4 and 2a). In each case, the GTP-bound form of the GTPase promotes association between two macromolecules and this association triggers (conditional) hydrolysis of the bound GTP. By analogy with kinetic proof-reading, the GTPase step in the Sec4 cycle may monitor appropriateness of the 'fit' between structural determinants on the donor vesicle and binding sites on putative docking proteins in the acceptor membrane.

At present the experimental evidence supporting this interpretation, summarized in Table 3, is suggestive but not compelling. Sec4 mutations do prevent secretory vesicles from attaching to the plasma membrane, and the Sec4 protein does cycle between cytoplasm, secretory vesicle, and plasma membrane^{97,108}, probably in the direction proposed²⁴. Future experiments will need to determine whether the plasma membrane contains Sec4 GAP activity, as Fig. 4 implies, as a preliminary step towards testing the most critical element of the hypothesis, that is, the postulated link between conditional hydrolysis of GTP and 'correct' binding of donor vesicle to the acceptor membrane.

The combination of genetic and biochemical analysis has already achieved unusually rapid progress in our understanding

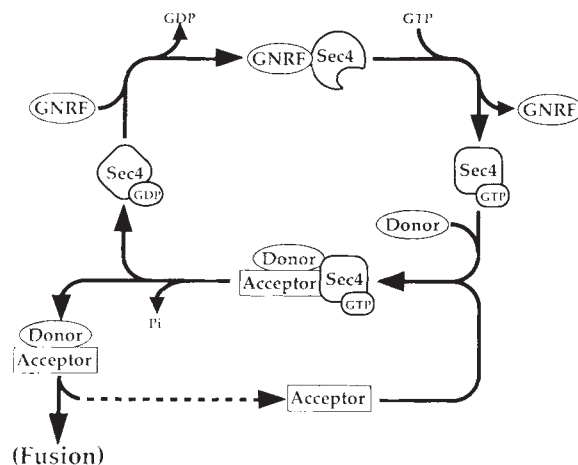


FIG. 4 Postulated GTPase cycle of Sec4 protein in *S. cerevisiae*. GNRF, Guanine nucleotide release protein; Donor, binding site on secretory vesicle; Acceptor, binding site on plasma membrane, recognized by Donor and Sec4.

of Sec4, whether or not future experiments precisely confirm the hypothesis shown in Fig. 4. Accessibility of the secretory pathway in *S. cerevisiae* to genetic manipulation will also make it easier to identify and characterize components of the vesicular transport machine that interact with Sec4. A potential target of Sec4, encoded by the *SEC15* gene, has recently been identified¹⁰⁹.

Targeting of nascent polypeptide chains

The newest GTPases on the block mediate targeting of nascent polypeptides to the ER membrane; this is the first step in a process that translocates proteins into the ER. One key component of the targeting machinery is the signal recognition particle (SRP), a protein-RNA complex that binds signal sequences of nascent polypeptides. SRP, bound to the polypeptide-ribosome complex, then binds to an SRP receptor (SRP-R) on the ER membrane, thereby initiating translocation of the nascent chain. This process is GTP-dependent¹¹¹. So far three proteins with amino-acid sequences characteristic of the GTPase superfamily have been found, including a 54K subunit of SRP^{14,15}, and the α^{17} and β (J. Miller and P. Walter, personal communication) subunits of SRP-R. Distinctive features in the amino-acid sequences of these three proteins, conserved between yeast and mammals¹⁶, indicate that they all belong to a new family of GTPases.

Although the precise biochemical functions of these putative GTPases are not yet defined, discovery of homologous proteins in *S. cerevisiae* and *Schizosaccharomyces pombe*¹⁶ suggests that answers will soon be forthcoming. For the moment it is easy to imagine, by analogy with the GTPases involved in ribosomal protein synthesis, that the GTPases in SRP and SRP-R promote and monitor the accuracy of successive steps in the complicated process of protein translocation.

Future questions

Our knowledge of most GTPases, except for those involved in protein synthesis, is less than ten years old. Investigations during the coming decade will refine and extend our understanding of GTPase functions in signal transduction, direction of vesicular traffic, and regulation of proliferation. Almost as certainly, the next ten years will also uncover new applications of the highly adaptable GTPase switch, applications that may compare in ubiquity and importance to those described in this review.

It is a safe bet, moreover, that the examples of EF-Tu, signaling G proteins, and p21^{ras} do not exhaust the possible arrangements of GTPases in regulatory circuits. Recently discovered direct interactions between GTPases—for example, a GTP-activated GNRP for eukaryotic IF2¹¹², G_i-G_i oligomers in retinal phototransduction¹¹³⁻¹¹⁵, and the multiple GTP-binding subunits of the SRP-R—strongly hint at a higher order of potential complexity. Like tubulin in a microtubule, individual GTPase molecules in a larger complex may turn out to modulate one another's rates of GTP hydrolysis, as well as affinities for guanine nucleotide and other protein ligands.

Finally, the GTPase cycle itself may easily be made to stand on its head, at least in theory: no obvious rules forbid a GTPase cycle in which, for example, a GDP-bound protein serves as the 'active' form of the GTPase, a ligand-activated GAP triggers its formation, and an GNRP inactivates it by converting into an inactive GTP-bound state.

Fanciful notions? Perhaps. We can only be certain that GTPases will continue to surprise us. □

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- Jurnak, F. *Science* **230**, 32-36 (1985).
- La Cour, T. F. M., Nyborg, J., Thirup, S. & Clark, B. F. C. *EMBO J.* **4**, 2385-2388 (1985).
- de Vois, A. M. *et al. Science* **239**, 888-893 (1988).
- Pai, E. F. *et al. Nature* **341**, 209-214 (1989).
- Milburn, M. V. *et al. Science* **247**, 939-945 (1990).
- Brünger, A. T. *et al. Proc. natn. Acad. Sci. U.S.A.* **87**, 4849-4853 (1990).
- Kaziro, Y. *Biochim. biophys. Acta* **505**, 95-127 (1978).
- Thompson, R. C. *Trends biochem. Sci.* **13**, 91-93 (1988).
- Neer, E. J. & Clapham, D. E. *Nature* **333**, 129-134 (1988).
- Stryer, L. & Bourne, H. R. *Rev. cell. Biol.* **2**, 391-419 (1986).
- Birnbaumer, L. *et al. Kidney Internat. (Suppl.)* **32**, S14-S37 (1987).
- Freissmuth, M., Caset, P. J. & Gilman, A. G. *FASEB J.* **3**, 2125-2131 (1989).
- Ross, E. M. *Neuron* **3**, 141-152 (1989).
- Romisch, K. *et al. Nature* **340**, 478-482 (1989).
- Bernstein, H. D. *et al. Nature* **340**, 482-486 (1989).
- Hann, B. C., Poritz, M. A. & Walter, P. *J. Cell Biol.* **109**, 3223-3230 (1989).
- Lauffer, L. *et al. Nature* **318**, 334-338 (1985).
- Barbacid, M. A. *Rev. Biochem.* **56**, 779-827 (1987).
- McCormick, F. *Cell* **56**, 5-8 (1989).
- Salminen, A. & Novick, P. *J. Cell* **49**, 527-538 (1987).
- Bacon, R. A., Salminen, A., Ruohola, H., Novick, P. & Ferro-Novick, S. *J. Cell Biol.* **109**, 105-1022 (1989).
- Schmitt, H. D., Wagner, P., Pfaff, E. & Gallwitz, D. *Cell* **47**, 401-412 (1986).
- Segev, N., Mulholland, J. & Botstein, D. *Cell* **52**, 915-924 (1988).
- Bourne, H. R. *Cell* **53**, 669-671 (1988).
- Masters, S. B., Stroud, R. M. & Bourne, H. R. *Protein Engng* **1**, 47-54 (1986).
- Heidman, W. & Bourne, H. R. in *G Proteins* (eds Birnbaumer, L. & Iyengar, R.) 17-40 (Academic, San Diego, 1990).
- Gibbs, J. B. *et al. in ADP-ribosylating Toxins and G Proteins, Insights into Signal Transduction* (eds Moss, J. & Vaughan, M.) 381-396 (American Society for Microbiology, Washington DC, 1990).
- Price, S. R., Barber, A. & Moss, J. in *ADP-ribosylating Toxins and G Proteins, Insights into Signal Transduction* (eds Moss, J. & Vaughan, M.) 397-424 (American Society for Microbiology, Washington DC, 1990).
- Trahey, M. & McCormick, F. *Science* **238**, 542-545 (1987).
- Gilman, A. G. *Rev. Biochem.* **56**, 615-649 (1987).
- Thompson, R. C., Dix, D. B. & Karim, A. M. *J. Biol. Chem.* **261**, 4868-4874 (1986).
- Fong, H. K. W., Yoshimoto, K. K., Eversole-Cire, P. & Simon, M. I. *Proc. natn. Acad. Sci. U.S.A.* **85**, 3066-3070 (1988).
- Matsuoka, M., Itoh, H., Kozasa, T. & Kaziro, Y. *Proc. natn. Acad. Sci. U.S.A.* **85**, 5384-5388 (1988).
- Strathmann, M., Wilkie, T. M. & Simon, M. I. *Proc. natn. Acad. Sci. U.S.A.* **86**, 7407-7409 (1989).
- Bourne, H. R. *Cold Spring Harbor Symp. quant. Biol.* **53**, 1019-1031 (1988).
- Bourne, H. R. & DeFranco, A. L. in *Oncogenes and the Molecular Origins of Cancer* (ed. Weinberg, R.) 97-124 (Cold Spring Harbor Laboratory, New York, 1989).
- Stryer, L. *Rev. Neurosci.* **9**, 87-119 (1986).
- Casey, P. J. & Gilman, A. G. *J. Biol. Chem.* **263**, 2577-2580 (1988).
- Graziano, M. & Gilman, A. G. *J. Biol. Chem.* **264**, 15475-15482 (1989).
- Landis, C. A. *et al. Nature* **340**, 692-696 (1989).
- Dietzel, C. & Kurjan, J. *Cell* **50**, 1001-1010 (1987).
- Nakafuku, M., Itoh, H., Nakamura, S. & Kaziro, Y. *Proc. natn. Acad. Sci. U.S.A.* **84**, 2140-2144 (1987).
- Miyajima, I. *et al. Cell* **50**, 1011-1019 (1987).
- Whiteway, M. *et al. Cell* **56**, 457-477 (1989).
- Bourne, H. R., Coffino, P. & Tomkins, G. M. *Science* **187**, 750-752 (1975).
- Harris, B. A., Robishaw, J. D., Mumby, S. M. & Gilman, A. G. *Science* **229**, 1274-1277 (1985).
- Robishaw, J. D., Russell, D. W., Harris, B. A., Smigel, M. D. & Gilman, A. G. *Proc. natn. Acad. Sci. U.S.A.* **83**, 1251-1255 (1986).
- Miyajima, I., Arai, K. I. & Matsumoto, K. *Molec. cell. Biol.* **9**, 2289-2297 (1989).
- Bourne, H. R. *Nature* **337**, 504-505 (1989).
- Birchmeier, C., Broek, D. & Wigler, M. *Cell* **43**, 615-621 (1985).
- Bar-Sagi, D. & Feramisco, J. R. *Cell* **42**, 841-848 (1985).
- Satoh, T., Nakamura, S. & Kaziro, Y. *Molec. cell. Biol.* **7**, 4553-4556 (1987).
- John, J., Frech, M. & Wittinghofer, A. *J. Biol. Chem.* **263**, 11792-11799 (1988).
- Neal, S. E., Eccleston, J. F., Hall, A. & Webb, M. R. *J. Biol. Chem.* **263**, 19718-19722 (1988).
- West, M., Kung, H.-F. & Kamata, T. *FEBS Lett.* **259**, 245-248 (1990).
- Wolfman, A. & Macara, I. G. *Science* **248**, 67-69 (1990).
- Downward, J., Riehl, R., Wu, L. & Weinberg, R. A. *Proc. natn. Acad. Sci. U.S.A.* **87**, 5998-6002 (1990).
- Vogel, U. S. *et al. Nature* **335**, 90-93 (1988).
- Trahey, M. *et al. Science* **242**, 1697-1700 (1988).
- Tabaka, K. *et al. Cell* **60**, 803-807 (1990).
- Sigal, I. S. *et al. Proc. natn. Acad. Sci. U.S.A.* **83**, 952-956 (1986).
- Walter, M., Clark, S. G. & Levinson, A. D. *Science* **233**, 649-652 (1986).
- Feig, L. A. & Cooper, G. M. *Molec. cell. Biol.* **8**, 2472-2478 (1988).
- Ballester, R. *et al. Cell* **59**, 681-686 (1989).
- Zhang, K. *et al. Nature* **346**, 719-723 (1990).
- Downward, J., Graves, J. D., Warne, P. H., Rayter, S. & Cantrell, D. A. *Nature* **346**, 719-723 (1990).
- Gibbs, J. B., Schaber, M. D., Schofield, T. L., Scolnick, E. M. & Sigal, I. S. *Proc. natn. Acad. Sci. U.S.A.* **86**, 6630-6634 (1989).
- Gibbs, J. B. & Marshall, M. S. *Microbiol. Rev.* **53**, 171-185 (1989).
- Hall, A. *Cell* **61**, 921-923 (1990).
- Yatani, A., Okabe, K., Polakis, P., McCormick, F. & Brown, A. M. *Cell* **61**, 769-776 (1990).
- Field, J., Broek, D., Kataoka, T. & Wigler, M. *Molec. cell. Biol.* **7**, 2128-2183 (1987).
- Broek, D. *et al. Cell* **41**, 763-769 (1985).
- Wigler, M. *et al. Cold Spring Harbor Symp. quant. Biol.* **53**, 649-655 (1988).
- Toda, T., Cameron, S., Sass, P., Zoller, M. & Wigler, M. *Cell* **50**, 277-287 (1987).
- Camomis, J. H. *et al. EMBO J.* **5**, 375-380 (1986).
- Broek, D. *et al. Cell* **48**, 789-799 (1987).
- Powers, S., O'Neill, K. & Wigler, M. *Molec. cell. Biol.* **9**, 390-395 (1989).
- Créchet, J.-B. *et al. Science* **248**, 866-868 (1990).
- Ellis, C., Moran, M., McCormick, F. & Pawson, T. *Nature* **343**, 377-381 (1990).
- Molloy, C. J. *et al. Nature* **342**, 711-714 (1989).
- Kaplan, D. R., Morrison, D. K., Wong, G., McCormick, F. & Williams, L. T. *Cell* **61**, 125-133 (1990).
- Kazlauskas, A., Ellis, C., Pawson, T. & Cooper, J. A. *Science* **247**, 1578-1581 (1990).
- Xu, G. *et al. Cell* **62**, 599-608 (1990).
- Garrett, M. D., Self, A. J., van Oers, C. & Hall, A. *J. Biol. Chem.* **264**, 10-13 (1989).
- Frech, M. *et al. Science* **249**, 169-171 (1990).
- Hata, Y. *et al. J. Biol. Chem.* **265**, 7104-7107 (1990).
- Kikuchi, A., Sasaki, T., Araki, S., Hata, Y. & Takai, Y. *J. Biol. Chem.* **264**, 9133-9136 (1989).
- Kitayama, H., Sugimoto, Y., Matsuzaki, T., Ikawa, Y. & Noda, M. *Cell* **56**, 77-84 (1989).
- Sasaki, T. *et al. J. Biol. Chem.* **265**, 2333-2337 (1990).
- Ueda, T., Kikuchi, A., Ohga, N., Yamamoto, J. & Takai, Y. *J. Biol. Chem.* **265**, 9373-9380 (1990).

91. Matsui, Y. *et al. Molec. cell. Biol.* **10**, 4116–4122 (1990).
92. Araki, S., Kikuchi, A., Hata, Y., Isomura, M. & Takai, Y. *J. biol. Chem.* **265**, 13007–13015 (1990).
93. Gutierrez, L., Magee, A. I., Marshall, C. J. & Hancock, J. F. *EMBO J.* **8**, 1093–1098 (1989).
94. Hancock, J. F., Magee, A. I., Childs, J. E. & Marshall, C. J. *Cell* **57**, 1167–1177 (1989).
95. Clarke, S., Vogel, J. P., Deschenes, R. J. & Stock, J. *Proc. natn. Acad. Sci. U.S.A.* **85**, 4643–4647 (1988).
96. Willumsen, B. M., Christensen, A., Hubbert, N. L., Papageorge, A. G. & Lowy, D. R. *Nature* **310**, 583–586 (1984).
97. Walworth, N. C., Goud, B., Kabacell, A. K. & Novick, P. *J. EMBO J.* **8**, 1685–1693 (1989).
98. Molenaar, C. M. T., Prange, R. & Gallwitz, D. *EMBO J.* **7**, 971–976 (1988).
99. Maltese, W. A., Sheridan, K. M., Repko, E. M. & Erdman, R. A. *J. biol. Chem.* **265**, 2148–2155 (1990).
100. Backlund, P. S. & Aksamit, R. R. *J. biol. Chem.* **263**, 15864–15867 (1988).
101. Melancon, P. *et al. Cell* **51**, 1053–1062 (1987).
102. Mayorga, L. S., Diaz, R. & Stahl, P. D. *Science* **244**, 1475–1477 (1989).
103. Mayorga, L. S., Diaz, R., Colombo, M. I. & Stahl, P. D. *Cell Reg.* **1**, 113–124 (1989).
104. Ruohola, H., Kabacell, A. K. & Ferro-Novick, S. *J. Cell Biol.* **107**, 1465–1476 (1988).
105. Baker, D., Hicke, L., Rexach, M., Schleyer, M. & Schekman, R. *Cell* **54**, (1988).
106. Schmitt, H. D., Puzicha, M. & Gallwitz, D. *Cell* **53**, 635–647 (1988).
107. Haubruck, H., Prange, R., Vorgias, C. & Gallwitz, D. *EMBO J.* **8**, 1427–1432 (1989).
108. Goud, B., Salminen, A., Walworth, N. C. & Novick, P. *J. Cell* **53**, 753–768 (1988).
109. Salminen, A. & Novick, P. *J. Cell Biol.* **109**, 1023–1036 (1989).
110. Chavrier, P., Parton, R. G., Hauri, H. P., Simons, K. & Zerial, M. *Cell* **62**, 317–329 (1990).
111. Connolly, T. & Gilmore, R. *Cell* **57**, 599–610 (1989).
112. Dholakia, J. N. & Wahba, A. J. *J. biol. Chem.* **264**, 546–550 (1989).
113. Wessling-Resnick, M. & Johnson, G. L. *J. biol. Chem.* **262**, 12444–12447 (1987).
114. Wessling-Resnick, M. & Johnson, G. L. *J. biol. Chem.* **262**, 3697–3705 (1987).
115. Hingorani, V. N., Tobias, D. T., Henderson, J. T. & Ho, Y. K. *J. biol. Chem.* **263**, 6916–6926 (1988).
116. Reed, R. R. *Cell* **60**, 1–2 (1990).
117. Fischer, V. *et al. Proc. natn. Acad. Sci. U.S.A.* **87**, 1988–1992 (1990).
118. Kim, S., Kikuchi, A., Mizoguchi, A. & Takai, Y. *Molec. Brain Res.* **6**, 167–176 (1989).
119. Mizoguchi, A. *et al. J. biol. Chem.* **265**, 11872–11879 (1990).
120. Darchen, F. *et al. Proc. natn. Acad. Sci. U.S.A.* **87**, 5692–5696 (1990).
121. Sekine, A., Fujiwara, M. & Narumiya, S. *J. biol. Chem.* **264**, 8602–8605 (1989).
122. Didsbury, J., Weber, R. F., Bokoch, G. M., Evans, T. & Snyderman, R. *J. biol. Chem.* **264**, 16378–16382 (1989).
123. Chardin, P. *et al. EMBO J.* **8**, 1087–1092 (1989).
124. Kikuchi, A., Yamamoto, K., Fujita, T. & Takai, Y. *J. biol. Chem.* **263**, 16303–16308 (1988).
125. Bender, A. & Pringle, J. R. *Proc. natn. Acad. Sci. U.S.A.* **86**, 9976–9980 (1989).
126. Johnson, D. I. & Pringle, J. R. *J. Cell Biol.* (in the press).
127. Sewell, J. L. & Kahn, R. A. *Proc. natn. Acad. Sci. U.S.A.* **85**, 4620–4624 (1988).
128. Kahn, R. A. & Gilman, A. G. *J. biol. Chem.* **261**, 7906–7911 (1986).
129. Sanders, D. A. *Cell Growth Diff.* **1**, 251–258 (1990).

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ARTICLES

Rapid dynamical evolution of giant comet Chiron

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Numerical simulation of orbits like that of the giant comet Chiron show a distinct asymmetry between past and future. Simulations extending $\pm 100,000$ years from the present suggest that on this time-scale, Chiron is about twice as likely to have been a short-period comet at some time in the past as to become one in the future. The mean half-life for such evolution is ~ 0.2 Myr, much less than the ~ 1 -Myr lifetime for ejection from the Solar System, implying that Chiron may have been a short-period comet in the past, and will probably become one in the future.

OBJECT Chiron, discovered in 1977 by Kowal and classified as a minor planet¹ (1977UB $\equiv$ 2060 Chiron), has frequently been regarded as a comet. Its orbit (see Table 1) lies entirely beyond Jupiter, but it crosses Saturn and is known to be chaotic^{2,3}. Interest in Chiron has grown following its gradual brightening and the detection of nebulosity from an asymmetric cometary coma^{4–6}. The diameter, assuming a geometric albedo of 0.1 and an absolute visual magnitude of 6.7, is ~ 200 km^{7,8}. Comet P/Chiron, as it presumably is, is the largest known, with a mass $\sim 10^4$ times that of comet Halley. The origin and history of Chiron is thus of special interest, both in relation to the origin of short-period comets and to questions concerning the evolution of so-called 'giant' comets and their possible interaction with the Earth^{9–15}.

Our study extends previous work^{2,3} by using a more realistic model of the outer Solar System and by integrating a larger sample of Chiron-like orbits forwards and backwards from the present epoch for a longer time. We consider the evolution of 83 orbits for ± 0.1 Myr. Over a third (37%) of our ensemble experienced a short-period phase of evolution (with period $P < 20$ yr) during the past 0.1 Myr and 23% evolved to such

orbits in the future. Contrary to suggestions^{3,4,8,16} that Chiron is a pristine object evolving for the first time towards a short-period orbit, we find that the probability of Chiron having already been a short-period comet is high. The ejection half-life is $t_{1/2,e} \approx 1.1$ – 1.4 Myr (in good agreement with the value given in ref. 17), whereas the half-life for Chiron to evolve into a short-period orbit is $t_{1/2,s} \approx 0.14$ – 0.26 Myr. (The half-life for transfer to Jupiter's control is 0.11 – 0.19 Myr.) Were Chiron first injected into its present orbit $t \approx 1$ – 100 Myr ago (consistent with the presence of volatiles and observed outgassing¹⁸), the probability of it never having been a short-period comet is $2^{-t/t_{1/2,e}} \leq 3\%$. The *a priori* probability of Chiron not having previously been a short-period comet is $t_{1/2,s}/(t_{1/2,e} + t_{1/2,s}) \approx 10\%$, similar to the estimate² of the probability of Chiron being ejected by Saturn before transfer to the control of Jupiter.

Here we present the dynamical results of our investigation, although we emphasize that Chiron's past evolution has potentially important implications for studies in other fields, including those of the climatological and geophysical record^{9,12,14,15}. Our results are consistent with the suggestion^{11,19} that Chiron or some Chiron-like body, may be the progenitor of the Taurid complex of Earth-crossing objects, and there are also important links with the origin of short-period comets^{20,21} and the inter-relationship between comets, asteroids and the zodiacal cloud^{16,22,23}. These aspects of Chiron's possible past history will be discussed elsewhere.

Long-term evolution

Chiron's chaotic motion (like that of many planet-crossing bodies^{24,25}) means that it is not possible accurately to predict its past or future orbit: any slight variation in the starting elements, in the type or precision of the integrator, or in the model adopted for the Solar System eventually produces a different outcome. The orbital elements of Chiron are not known to arbitrary precision, nor is it known the extent to which non-gravitational forces affect the motion. Moreover, even the best Solar System models fail to predict the observed positions of the outer planets to better than about one arcsecond over a

20-year period²⁶. A single calculation of Chiron's long-term motion thus has limited validity, so we have considered an ensemble^{2,3}.

The elements of 2060 Chiron are summarized in Table 1 together with rounded versions that define the central orbit of our ensemble (CH00). Comparing this orbit with a previous determination²⁷ integrated forwards to the same epoch, allows us to evaluate the present uncertainty in the elements. We estimate $\Delta a \approx 10^{-4}$ AU, $\Delta e \approx 10^{-5}$ and $\Delta i \approx \Delta \Omega \approx \Delta M \approx 10^{-4}$ deg, indicating a typical range of about ± 1 in the last digit of the elements defining CH00. An array of 81 representative 'Chirons' was then obtained by fixing ω and M at their CH00 values, and changing the other elements (a, e, i, Ω) by one unit or zero in the last digit according to a cyclic pattern to give all possible combinations. Object CH41 has identical elements to CH00, making a total of 83 'Chirons' in all. Six representative cases are summarized in Table 1.

These 83 objects were integrated in 7 groups of 11 (2060 Chiron, CH00, CH01, ..., CH09) and one of 6. The model Solar System included the mutual interactions of Jupiter, Saturn, Uranus and Neptune, and had the masses of the inner planets added to that of the Sun. The initial positions, velocities and masses were taken from the Jet Propulsion Laboratories ephemeris DE118, and the resulting four-planet Solar System (with the Chirons treated as test particles) was integrated backwards and forwards for $\pm 100,000$ years using Everhart's RADAU integrator²⁸ to fifteenth order with tolerance set at 10^{-12} and an initial step size of 40 days. This program adjusts the step size in each time step to maintain the accuracy of the integration for all the objects in a given batch. We found that the results for objects in a single batch were perfectly reproducible, but that initially identical orbits calculated as part of two different groups (for example, CH00 and CH41) soon evolved in quite different ways. This was because of the different sequence of variable step sizes used in each group. Such behaviour is to be expected for chaotic orbits that suffer repeated close encounters to the major planets²⁹. The accuracy of the integrator has been thoroughly tested by forwards and backwards calculations of orbits that experience only distant encounter²⁵.

TABLE 1 Adopted elements of representative Chirons

Object	a (AU)	e	i (deg)	Ω (deg)
2060 Chiron	13.6678867	0.3797169	6.93242	208.63174
CH00/CH41	13.6679	0.37972	6.9324	208.6317
CH53	13.6679	0.37973	6.9325	208.6317
CH61	13.6680	0.37971	6.9325	208.6316
CH63	13.6680	0.37971	6.9325	208.6318

Orbital elements for 2060 Chiron and representative sample orbits CH nn . These are for epoch 1987 July 24.0 (JD 2447000.5) and equinox 1950.0. The argument of perihelion ω and mean anomaly at epoch M of Chiron are 339.35571° and 298.93237° ; for the sample orbits CH nn these are rounded to 339.356° and 298.932° , respectively. The perihelion distance is $q = 8.478$ AU and the period is 50.53 yr.

Range of orbital types

Figure 1 shows the evolution of semi-major axis and perihelion distance for four representative orbits. Plots such as these allowed the time spent in different orbital states to be determined, together with the duration of individual mean motion resonances classified as 'stable'. We adopted a simple classification scheme using the orbital period (short-period, 'SP'; intermediate-period, 'IP'; long-period, 'LP'; or parabolic, 'Para'), the relative stability ('V' for a variable Chiron-like evolution, 'S' for more stable motion in temporary resonance), and the controlling planet (J, S, U or N to denote motion under the control of Jupiter, Saturn, Uranus or Neptune, respectively). The notation SP(J), for example, denotes a short-period orbit under the control of Jupiter.

Although exhibiting a wide range of behaviour most evolutions qualitatively resembled those in Fig. 1. There were frequent cases of intermediate-period variable behaviour under the control of Saturn (like Chiron's present orbit), whereas others evolved to short period under the control of Jupiter (as in CH41). Some spent a considerable time as intermediate-period objects under the control of Jupiter, others exhibited long periods of near-resonant behaviour governed by low-order mean motion resonances with Saturn. Such behaviour is illustrated by CH63 which was dominated in the past by the 1/3

FIG. 1 The evolution of four representative Chirons for $\pm 100,000$ years from the present epoch. The solid and dotted lines denote the semi-major axis and perihelion distance, respectively, and the horizontal dashed lines show the semi-major axes that conventionally separate long-period ($P > 200$ yr), intermediate-period ($20 < P \leq 200$ yr) and short-period ($P \leq 20$ yr) orbits. Plots such as these, together with subsidiary data from plots of the eccentricity and inclination, the Tisserand parameter $T_p = (a_p/a) + 2[(a/a_p)(1-e^2)]^{1/2} \cos(i-i_p)$ with respect to each planet (where subscript p refers to the planet), and the distribution of close planetary encounters, enabled each orbit to be classified as a function of time in terms of period, relative stability and the controlling planet.

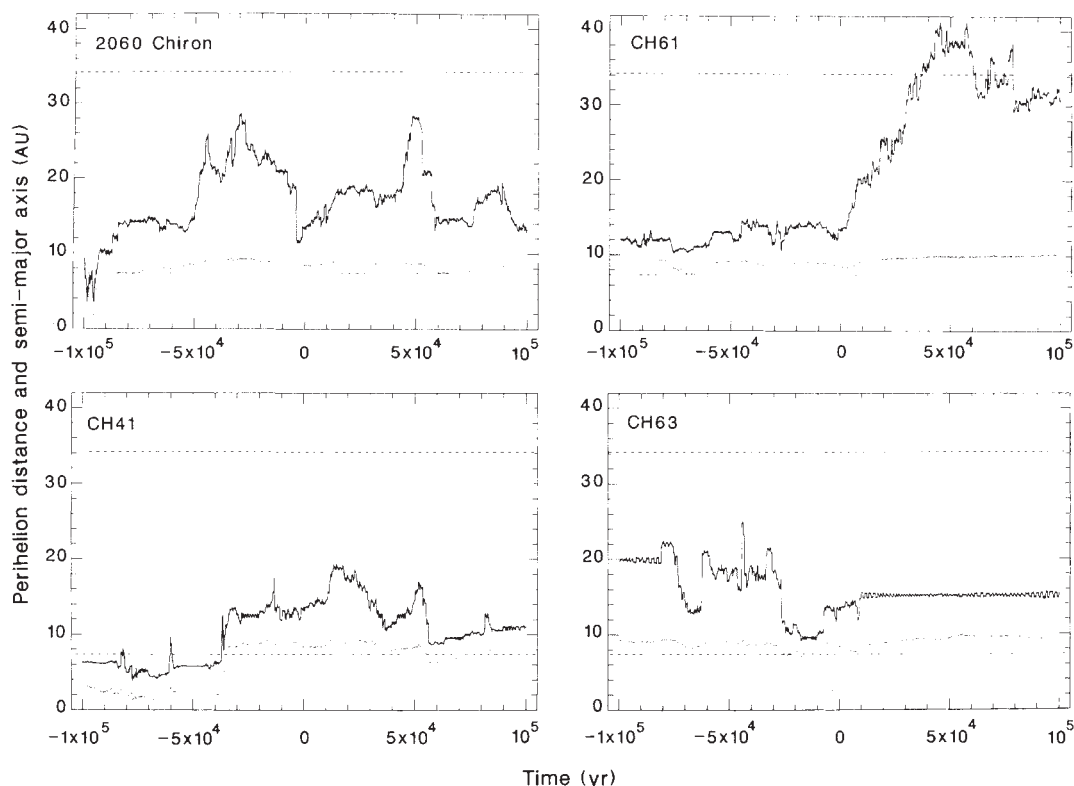


TABLE 2 Frequency distribution of instantaneous orbital types

Time from present (yr)	SP(J)		IP(J)		Number of Chirons in each orbital type		IPV(S)		IP(U)		LP		Para	
	past	future	past	future	past	future	past	future	past	future	past	future	past	future
0	0	0	0	0	0	0	83	83	0	0	0	0	0	0
10,000	0	0	6	0	14	9	62	74	1	0	0	0	0	0
20,000	3	1	5	2	21	11	52	68	1	0	1	1	0	0
30,000	5	0	6	6	20	21	49	56	1	0	2	0	0	0
40,000	7	4	6	6	19	15	45	54	1	0	5	4	0	0
50,000	3	2	10	7	18	15	45	53	1	0	5	6	1	0
60,000	5	4	11	6	15	19	41	46	2	0	8	8	1	0
70,000	4	4	14	5	18	20	34	44	3	1	8	8	2	1
80,000	7	3	15	8	17	14	33	48	3	2	5	7	3	1
90,000	6	0	14	9	16	14	32	43	3	3	8	11	4	3
100,000	7	3	12	7	12	10	36	45	3	3	8	11	5	4
% Time in each orbital type	5.0	2.4	11.4	7.2	21.1	18.0	53.2	65.1	2.1	0.9	5.5	5.6	1.6	0.8

The last line gives the percentage of the total time (100,000 yr) spent in these orbital types for the whole ensemble.

resonance with Saturn and in the future by the 1/2 resonance. (In the 3/1 mean-motion resonance, for example, one revolution period of Chiron corresponds to three of the controlling planet, in this case Saturn.) CH61, on the other hand, gradually evolved towards longer period and larger perihelion distance, with a short interval of long-period behaviour. In general, bodies moving under the control of Jupiter were more common in the past. One such is CH53, illustrated in Fig. 2.

The long intervals of near-stable motion demonstrate the importance of low-order temporary resonances with either Saturn or Jupiter. Similar results have been described by Everhart^{30,31} and generally affect a wide range of planet-crossing orbits^{24,32-34}, even retrograde types like P/Halley³⁵. Combining the backwards and forwards integrations, the Jupiter resonances included the 1/2, 2/3 and 2/5 (occurring in 14, 11 and 11 cases, respectively, with a mean duration of the order of 5,000 years), whereas the most frequent Saturn resonances included the 1/2 (35 cases, mean duration 22,000 years), the 2/3 (27 cases, mean duration 15,000 years), the 1/1 and related high-order substates (38 cases, mean duration 6,000 years), and the 3/5 (14 cases, mean duration 8,000 years). A noticeable 11/19 resonance with Saturn occurred in 19 cases; this seems chiefly to be a characteristic of the near-future orbit, in the sense that it usually (but not exclusively) affects a few thousand years within about 6,000 years of the present epoch. Others have also noted the importance for Chiron of low-order temporary resonances with Saturn and related correlations in the oscillations of perihelion distance,

eccentricity and inclination¹.

The difference in this evolution from that expected on a 'random walk' hypothesis is remarkable, with variations in semi-major axis frequently occurring as discrete 'jumps' from one near-resonant state to another. Insofar as this behaviour is a function of both the direct and indirect perturbations of neighbouring planets, this result casts further doubt^{20,36} on the validity of previous attempts to calculate the capture times and capture probability of long-period comets into short-period orbits, whether using a standard Monte Carlo scheme or by increasing the planetary masses by a large factor^{37,38}. Neither the capture time nor the resonances are correctly handled by such procedures.

None of the orbits in Fig. 1 should be given greater weight than another, each representing only a possible trajectory along which the real Chiron might have passed. Nevertheless we may ask, statistically, whether the 83 sample Chirons show any difference between their past and future evolution. In this way we can address whether Chiron is more likely to be coming in from the Oort cloud for the first time or is a relatively 'old' comet, having previously been an ordinary Jupiter-family short-period comet.

Probable short-period history

The data in Table 2 indicate a greater likelihood for Chiron being under the control of Jupiter in the past than in the future, and at any one time about 20% of the objects are moving in

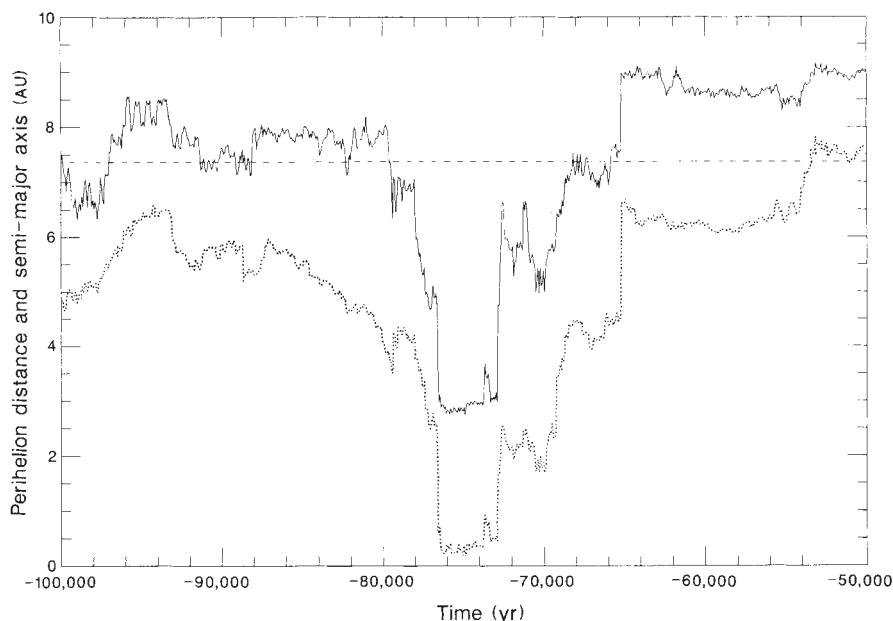


FIG. 2 The first 50,000 years of object CH53. It became an Earth-crossing comet with aphelion close to Jupiter's perihelion around 75,000 yr ago, and then evolved under the control of Saturn eventually to a typical IPV(S) orbit, like the present Chiron. If conventional formulae for cometary mass loss are used, the total dust ejected during the Earth-crossing phase of evolution would have amounted to 10^{17} – 10^{18} kg.

relatively stable orbits under the control of Saturn. The four or five objects ejected into parabolic orbits in 10^5 years imply a half-life for ejection in the range 1.1–1.4 Myr, in good agreement with previous results^{2,17}.

Although the dynamical lifetime for Chiron-like orbits is of the order of 1 Myr, a significant percentage of the sample had short-period orbits or were moving under the control of Jupiter within a few tens of thousands of years from now. This result, indicating rapid dynamical evolution, is shown in Fig. 3. Overall, 45% of our sample came under Jupiter's control within the past 0.1 Myr, but only 30% did so in the future. Combining both sets of data, 61% came under the control of Jupiter within 0.1 Myr of the present epoch, and 52% became short-period comets. The half-life for Chiron to have evolved from a short-period orbit ($P < 20$ yr) in the past is ~ 0.14 Myr, whereas that for the future is 0.26 Myr. Neglecting the past-future asymmetry, the average half-life for such an evolution is ~ 0.2 Myr.

The detected asymmetry in Chiron's present orbit is relatively weak, although opposite to that found previously³ and going against the current tendency^{4,8,16} to view Chiron as a pristine object. Still, we believe the asymmetry is real, probably caused by the time of the first close encounter with Saturn. As shown in Fig. 1, this occurs nearer in the past than the future, at times of the order of 1,700 and 12,000 years, respectively, imposing a bias which persists for the whole evolution. Nevertheless, the more important point is that bodies in Chiron-like orbits evolve extremely rapidly both towards and away from short-period orbits, on a timescale measured in thousands or tens of thousands of years. Comets in Chiron-like orbits have a high probability of becoming temporary short-period comets and the results of our investigation provide insight into the detailed balance between different orbital types. Unless Chiron was recently (compared to the ejection timescale) injected into its present orbit, P/Chiron has presumably been a past short-period comet.

For example, if comets are injected into Chiron-like orbits at a rate R per unit time and dynamically ejected with a probability p_e per unit time per object and inserted into a short-period orbit with probability p_s per unit time per object, the steady-state number of such bodies is R/p_e , and $R/(p_e + p_s)$ bodies will never have experienced a short-period phase. The *a priori* probability of Chiron never having been a short-period comet is $p_e/(p_e + p_s) = t_{1/2,s}/(t_{1/2,e} + t_{1/2,s}) \approx 0.1$.

Implications

Our calculations show that bodies in Chiron-like orbits undergo rapid evolution between different orbital types, some becoming short-period ($P < 20$ yr) and some (like CH53) even Earth-crossing. Unless Chiron is exceptional (for example, in being

both unique and recently captured) either it or some other similarly large comet must at some time have circulated in the inner Solar System as a short-period comet. One cannot be sure that Chiron has experienced such an evolution (the probability during the past 10^5 years is only 37%), but given that Chiron is such a large comet it is interesting and important to explore possible consequences of such a history.

For example, we found that several members of our ensemble evolved into orbits resembling those of known near-Earth asteroids (such as 1986JK and 1987SL), some of which are close to the 5/2 resonance with Jupiter and can evolve rapidly on timescales of the order of 10^5 yr^{25,39–41}. Temporary jet-reaction forces owing to outgassing, collisions with debris in the asteroid belt, or even with comet-produced 'boulders'⁴², might cause them to decouple from Jupiter and thereby be placed in more-stable orbits.

Moreover, the zodiacal cloud seems not to be in a steady state^{19,43}, the observed cometary sources failing by about an order of magnitude to replenish the predicted losses. There may also be too many short-period comets to be explained by capture from an observed source flux. Although possible alternative explanations have been offered to overcome these difficulties (for example, that the zodiacal cloud is dominated by material originating in the asteroid belt⁴⁴, or that short-period comets come from a spherically symmetrical inner core of the Oort cloud³⁶ or a comet belt beyond Neptune³⁷), both might in principle be resolved by postulating injection of a giant comet into the inner Solar System about 10^4 years ago, and assuming that its subsequent evolution and decay^{10–12} produced many short-period comets and an enhanced zodiacal cloud.

The idea dates back many years¹⁵, but has been described as *ad hoc* because the progenitor has not been identified. The cometary nature of Chiron and its possible short-period history, however, puts the idea on a more secure footing. One criticism is that a random giant comet from the Oort cloud would probably not produce short-period comets with inclinations so closely confined to the ecliptic. Because Chiron has an inclination of only 7° , this point seems to be answered. Similarly, although P/Encke has frequently been linked to such an evolution^{11–13,45} (being associated with the Taurid meteor stream⁴⁶ and dominating the production of cometary dust), not only is dust output from P/Encke insufficient to maintain the zodiacal cloud in a steady state but its present orbit (with aphelion decoupled from Jupiter) is generally difficult to explain⁴⁷. If Chiron (or some other large comet) has previously circulated in a short-period orbit, however, it may be possible to argue^{11,12} that P/Encke and the associated bodies in the Taurid meteor stream complex were produced by ejection of material from the giant comet's outer layers. Ejection of P/Encke (or its progenitor) from Chiron

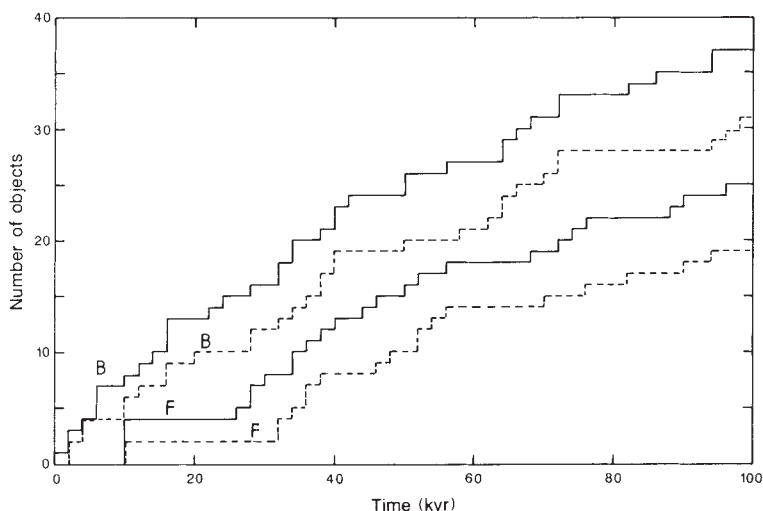


FIG. 3 The number of objects that temporarily came under the control of Jupiter as a function of time from the present (solid lines) or which temporarily became short-period comets (dashed lines), for both forwards (F) and backwards (B) integrations. Taking the ensemble of 83 objects together, the average Chiron spent 5% and 11.4% of the time in a short-period or intermediate-period orbit under the control of Jupiter during the past 0.1 Myr, and 2.4% and 7.2% of the time in such orbits in the future.

during an earlier short-period phase of evolution may provide an explanation for the otherwise extreme nature of the forces that have to be invoked to explain P/Encke's present orbit.

We emphasize that ejection of material from a giant comet during an active short-period phase of evolution is not *ad hoc*. The Sun-grazer family⁴⁸, thought to involve a comet originally of comparable size to Chiron⁴⁹, supports the notion that large comets may evolve by repeated fragmentation to produce discrete bodies of 'cometary' size, and several other comets are known to have common progenitors (such as P/Neujmin 3 and P/Van Biesbroeck⁵⁰, and comets Levy and Shoemaker-Holt⁵¹). Observations of P/Halley indicate the presence of kilometre-sized cometary 'building blocks' in the nucleus⁵², and comets of all types have been known to split or suffer large outbursts⁵³. At present, the cause of outbursts is not known, but plausible mechanisms include impacts by interplanetary boulders, close passages to the Sun or a major planet, rotational disruption, or even runaway 'explosive' reactions⁵⁴. A body the size of Chiron in a short-period orbit would experience impact events leading to kilometre-sized craters about once every 25 revolutions⁵⁵, and its rapid rotation ($P_R = 5.9178$ h; ref. 6) implies stresses of the order of $\rho d^2/P_R^2 \approx 10^5 \text{ N m}^{-2}$ (where ρ is the density and d the diameter), comparable with the internal strength of the Sun-grazer progenitor⁴⁹. With a diameter of 200 km for Chiron and a density of 1 g cm^{-3} , the rotational speed at the equator is $\sim 40\%$ of the escape velocity. A combination of these mechanisms could allow substantial fragments of material to leave the

nucleus despite the relatively high escape velocity ($\sim 70 \text{ m s}^{-1}$).

Thus, there is an important dynamical link between bodies in Chiron-like orbits and ordinary short-period comets. Whereas the half-life for Chiron to be ejected is in the range 1.1–1.4 Myr, the half-life to be transferred to an orbit with $P < 20$ yr is only 0.14–0.26 Myr. Unless Chiron was recently injected into its present orbit, there is a significant probability that it was previously a short-period comet. This suggests^{11,19} that debris from Chiron, or some other Chiron-like body, may have been instrumental in producing the stream of material in the planetary system known as the Taurid complex, comprising meteoroids, Encke's comet and possibly some 50–100 Earth-crossing asteroids¹⁵, with potentially important implications for Earth's climate.

The nature of Chiron's present orbit, one of transition between short-period and long-period comets, means that further studies of this and similarly chaotic orbits should provide important tests of cometary dynamics. Observations of its physical properties as it approaches perihelion in February 1996 may provide clues to its detailed evolution and composition, supporting or contradicting the proposed past history. Chiron plays a pivotal role in current theories of the formation and evolution of comets, and further observational and theoretical studies of this and related objects (such as 944 Hidalgo, P/Schwassmann-Wachmann 1) are eagerly awaited. Chiron should be considered a prime candidate to be explored by any cometary space mission planned for the next century. □

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- Kowal, C. T., Liller, W. & Marsden, B. G. *IAU Symp.* **81**, 245–250 (1979).
- Oikawa, S. & Everhart, E. *Astr. J.* **84**, 134–139 (1979).
- Scholl, H. *Icarus* **40**, 345–349 (1979).
- Hartmann, W. K., Tholen, D. J., Meech, K. J. & Cruikshank, D. P. *Icarus* **83**, 1–15 (1990).
- Everhart, J. *Science News* **137**, 244 (1990).
- Luu, J. X. & Jewitt, D. C. *Astr. J.* **100**, 913–932 (1990).
- Lebofsky, L. A., Tholen, D. J., Rieke, G. H. & Lebofsky, M. J. *Icarus* **60**, 532–537 (1984).
- Bus, S. J., Bowell, E., Harris, A. & Hewitt, A. V. *Icarus* **77**, 223–238 (1989).
- Hoyle, F. & Wickramasinghe, N. C. *Astrophys. Space Sci.* **53**, 523–526 (1978).
- Clube, S. V. M. & Napier, W. M. *Q. J. R. astr. Soc.* **23**, 45–66 (1982).
- Clube, S. V. M. & Napier, W. M. *Mon. Not. R. astr. Soc.* **211**, 953–968 (1984).
- Clube, S. V. M. & Napier, W. M. in *The Galaxy and the Solar System* (eds Smoluchowski, R. et al.) 260–285 (University of Arizona Press, Tucson, 1986).
- Clube, S. V. M. in *Catastrophes and Evolution* (ed. Clube, S. V. M.) 81–112 (Cambridge University Press, 1989).
- Wickramasinghe, N. C., Hoyle, F. & Rabilizirov, R. *Nature* **341**, 28 (1989).
- Bailey, M. E., Clube, S. V. M. & Napier, W. M. *The Origin of Comets* (Pergamon, 1990).
- Weissman, P. R., A'Hearn, M. F., McFadden, L. A. & Rickman, H. in *Asteroids II* (eds Binzel, R., Gehrels, T. & Matthews, M. S.) 880–920 (University of Arizona Press, Tucson, 1990).
- Wetherill, G. W. *Proc. lunar planet. Sci. Conf.* **6**, 1539–1561 (1975).
- Stern, S. A. *Publ. astr. Soc. Pacif.* **101**, 126–132 (1989).
- Olsson-Steel, D. *Mon. Not. R. astr. Soc.* **219**, 47–73 (1986).
- Bailey, M. E. & Stagg, C. R. *Icarus* **86**, 2–8 (1990).
- Bailey, M. E. in *Asteroids Comets Meteors III* (eds Lagerkvist, C.-I. et al.) 221–230 (Uppsala Observatory, 1990).
- Rickman, H. *IAU Colloq.* **83**, 149–172 (1985).
- Clube, S. V. M. *Phil. Trans. R. Soc. A* **323**, 421–436 (1987).
- Milani, A., Carpino, M., Hahn, G. & Nobili, A. M. *Icarus* **78**, 212–269 (1989).
- Hahn, G. & Lagerkvist, C.-I. *Celest. Mech.* **43**, 285–302 (1988).
- Seidemann, P. K. & Harrington, R. S. *Celest. Mech.* **43**, 55–68 (1988).
- Marsden, B. G. *IAU Circ. No.* 3151 (1977).
- Everhart, E. *IAU Colloq.* **83**, 185–202 (1985).
- Milani, A. & Nobili, A. M. *IAU Colloq.* **83**, 215–226 (1985).
- Everhart, E. *Astr. J.* **78**, 316–328 (1973).
- Everhart, E. *Astr. J.* **78**, 329–337 (1973).
- Milani, A., Carpino, M. & Marzari, F. *Icarus* (in the press).
- Carusi, A., Kresák, L., Perozzi, E. & Valsecchi, G. B. *Astr. Astrophys.* **187**, 899–905 (1987).
- Rickman, H. & Froeschlé, C. *Celest. Mech.* **43**, 243–263 (1988).
- Dvorak, R. & Kribbel, J. *Astr. Astrophys.* **227**, 264–270 (1990).
- Stagg, C. R. & Bailey, M. E. *Mon. Not. R. astr. Soc.* **241**, 507–541 (1989) and microfiche MN 241/1.
- Duncan, M., Quinn, T. & Tremaine, S. *Astrophys. J.* **328**, L69–L73 (1988).
- Quinn, T., Tremaine, S. & Duncan, M. *Astrophys. J.* **355**, 667–679 (1990).
- Hahn, G. & Rickman, H. *Icarus* **61**, 417–442 (1985).
- Yoshikawa, M. *Astr. Astrophys.* **213**, 436–458 (1989).
- Hahn, G., Lagerkvist, C.-I., Lundgren, M. & Dahlgren, M. in *Asteroids Comets Meteors III* (eds Lagerkvist, C.-I. et al.) 95–98 (Uppsala Observatory, 1990).
- Marsden, B. G. & Sekanina, Z. *Astr. J.* **76**, 1135–1151 (1971).
- Kresák, L. & Kresáková, M. in *Symp. on the Diversity and Similarity of Comets, ESA SP-278* (eds Rolfe, E. J. & Batrick, B.) 739–744 (European Space Agency, Noordwijk, 1987).
- Sykes, M. *Icarus* (in the press).
- Clube, S. V. M. & Asher, D. J. in *Asteroids Comets Meteors III* (eds Lagerkvist, C.-I. et al.) 275–280 (Uppsala Observatory, 1990).
- Whipple, F. L. & Hamid, S. E. *Helwan Obs. Bull.* **41**, 1–30 (1952).
- Kresák, L. in *Highlights of Astronomy Vol. 6* (ed. West, R. M.) 377–390 (1983).
- Marsden, B. G. *Astr. J.* **98**, 2306–2321 (1989).
- Öpik, E. J. *Irish Astr. J.* **7**, 141–161 (1966).
- Carusi, A., Perozzi, E. & Valsecchi, G. B. in *The Evolution of the Small Bodies of the Solar System* (eds Fulchignoni, M. & Kresák, L.) 191–201 (North-Holland, Amsterdam, 1987).
- Marsden, B. G. *Minor Planet Circ. No.* 13452 (1988).
- Möhlmann, D., Danz, M. & Börner, H. in *Symp. on the Diversity and Similarity of Comets, ESA SP-278* (eds Rolfe, E. J. & Batrick, B.) 487–492 (European Space Agency, Noordwijk, 1987).
- Hughes, D. W. *Q. J. R. astr. Soc.* **31**, 69–94 (1990).
- Bar-Nun, A. & Prialnik, D. *Adv. Space Res.* (in the press).
- Fernández, J. A. in *Asteroids Comets Meteors III* (eds Lagerkvist, C.-I. et al.) 309–312 (Uppsala University, 1990).

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Requirement for hsp70 in the mitochondrial matrix for translocation and folding of precursor proteins

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By analysis of a temperature-sensitive yeast mutant, a heat-shock protein in the matrix of mitochondria, mitochondrial hsp70 (Ssc1p), is found to be involved both in translocation of nuclear-encoded precursor proteins across the mitochondrial membranes and in (re)folding of imported proteins in the matrix.

THE biogenesis of mitochondrial proteins is a multistep process¹⁻³ in which precursor proteins synthesized in the cytosol are recognized by specific receptors on the mitochondrial surface. The precursors are inserted into the outer membrane and are translocated through contact sites between both mitochondrial membranes in an unfolded conformation. In the matrix, amino-terminal signal sequences (presequences) of the precursors are proteolytically cleaved. The imported proteins are (re)folded and are assembled into functional complexes. An important goal in the molecular analysis of organelle biogenesis is the identification and characterization of the components that are required for mitochondrial protein import. Stress proteins or heat shock proteins (hsps) are involved in several steps of protein transfer into mitochondria.

Hsps, so named because expression of most (but not all) is increased under various cellular stress conditions, have been grouped into several families according to similarities in their primary structure⁴. The largest hsp family is composed of proteins of relative molecular mass about 70,000 ($M_r \sim 70$ K) (hsp70s). Cytosolic hsp70s, termed Ssa1-4p in yeast⁵, stimulate translocation of precursor proteins into mitochondria and endoplasmic reticulum (ER), suggesting these hsp70s are involved in conferring transport-competence to precursors⁶⁻¹⁰. The 60K hsp family ('chaperonins') includes mitochondrial hsp60, chloroplast Rubisco-subunit binding protein and GroEL in *Escherichia coli*¹¹⁻¹⁴. By using transport intermediates of precursor proteins, hsp60 was shown to interact with imported proteins in an ATP-dependent reaction, thereby promoting the (re)folding and assembly of proteins, giving a first indication for a function of hsp60 as a 'foldase'^{15,16}. The experimental generation of translocation and folding intermediates of mitochondrial precursor proteins thus provides a powerful system for the analysis of two intriguing problems in cell biology, the mechanisms of intracellular protein sorting and the functions of heat shock proteins.

We recently reported that of the eight hsp70s that have been identified in the yeast *Saccharomyces cerevisiae*, one (Ssc1p) is located in mitochondria¹⁷. This protein performs an essential cellular function as disruption of *SSC1* results in lethality¹⁸. Mitochondrial hsp70s have also been found in several other organisms¹⁹⁻²². To study the function of Ssc1p we isolated a temperature-sensitive *ssc1* yeast mutant. As the mutant cells

accumulated the precursor forms of mitochondrial proteins, we analysed the assembly pathways of mitochondrial proteins in detail and found a dual role for Ssc1p: involvement in translocation of precursor proteins through mitochondrial contact sites and in folding of proteins in the mitochondrial matrix. We believe that the homologous hsp70s in prokaryotes, eukaryotic cytosol, ER and chloroplasts may have similar functions.

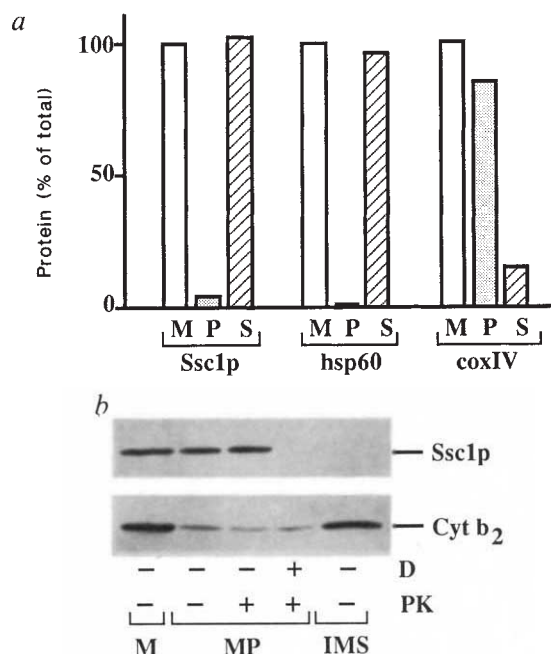


FIG. 1 Mitochondrial hsp70 (Ssc1p) is located in the matrix. *a*, Separation of membrane and soluble components. Mitochondrial protein (M, 30 μ g) and the fractional equivalents of membrane pellet (P) and soluble components (S) analysed by immunoblotting with antisera against: Ssc1p, hsp60 and subunit IV of cytochrome oxidase (coxIV). *b*, Formation of mitoplasts and release of components of the intermembrane space (IMS). Mitochondria (M, 30 μ g) or the fractional equivalents of mitoplasts (MP) or soluble components (IMS) released during mitoplast formation. PK, proteinase K; D, detergent (SDS). Antisera: Ssc1p, coxIV, cytochrome b_2 (Cyt b_2).

METHODS. Mitochondria were prepared from D273-10B cells grown in semi-synthetic media containing 3% glycerol and 2% lactate as described¹⁷. *a*, Mitochondria were subjected to sonication and the membrane components separated by centrifugation for 20 min at 48,000 r.p.m. in a Beckman TLA 100.3 rotor. *b*, Mitoplasts were prepared by diluting mitochondria (10 mg ml⁻¹ in 250 mM sucrose, 1 mM EDTA, 10 mM MOPS buffer, pH 7.2) fivefold in 10 mM Tris, pH 7.4. The mitoplasts were pelleted by centrifugation for 20 min at 37,000 r.p.m. in a Beckman TLA 100.3 rotor. The supernatant (the IMS fraction) was removed and the mitoplasts resuspended in the original volume (10 mg ml⁻¹ equivalent) of 0.1 M mannitol, 10 mM Tris pH 7.4. Aliquots were treated with 0.2 mg ml⁻¹ of proteinase K $\pm$ 0.2% SDS for 20 min at 0 °C. The indicated amounts of each fraction were separated by SDS-PAGE and subjected to immunoblot analysis. The filter-bound immunoglobulins were visualized by the use of secondary antibodies linked to alkaline phosphatase and a chromogenic substrate¹⁷.

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Ssc1p is localized in the matrix

To determine the submitochondrial localization of Ssc1p, antibodies specifically directed against Ssc1p were prepared. As the 11 carboxyl-terminal amino-acid residues of Ssc1p are not conserved in other hsp70s (ref. 17), a chemically synthesized peptide corresponding to these residues was used for immunization. The antibodies reacted with Ssc1p on two-dimensional SDS polyacrylamide gels¹⁷, but not with any other hsp70 (data not shown). Mitochondria were disrupted by sonication and the soluble and membrane fractions separated by centrifugation. Ssc1p was found in the soluble fraction, as was hsp60, a soluble matrix protein (Fig. 1a). As expected, the inner membrane protein subunit IV of cytochrome oxidase was present predominantly in the membrane fraction (Fig. 1a). To distinguish between a localization of Ssc1p in the intermembrane space or the matrix, the mitochondrial outer membrane was selectively broken, releasing most cytochrome *b*₂ from the intermembrane space (Fig. 1b). Ssc1p remained associated with the mitoplasts in a location protected from proteases. Ssc1p only became accessible to added protease on disruption of the inner mitochondrial membrane with detergent (Fig. 1b), as was found with other mitochondrial matrix proteins²³. We conclude that Ssc1p is a soluble protein of the mitochondrial matrix.

A temperature-sensitive *SSC1* mutant

To analyse the function of Ssc1p we isolated temperature-sensitive (ts) yeast mutants carrying mutations in *SSC1*. Strain BC100 (*GAL1::SSC1*), which carries an insertion mutation in the chromosomal copy of *SSC1* and a centromeric plasmid containing a wild-type *SSC1* gene fused to the glucose-repressible *GAL1* promoter, was used in the mutant screen (Fig. 2). As *SSC1* is an essential gene, the strain was maintained on galactose-based media to allow *SSC1* expression from the *GAL1* promoter. Another centromeric plasmid containing *SSC1* under the control of its own promoter was mutagenized *in vitro* with hydroxylamine and used to transform BC100 (*GAL1::SSC1*). Transformants were screened for growth on glucose-based media at 23 °C and 37 °C. Those permissive for growth at 23 °C,

but not 37 °C were candidates for strains carrying *ssc1* temperature-sensitive (ts) mutations. Transformants were also screened for growth at 37 °C on galactose-based media to eliminate *SSC1*-independent ts mutations resulting from the transformation procedure. Several tests were performed on the putative *SSC1* mutant plasmids to confirm that the mutation conferring temperature sensitivity was in the *SSC1* gene (see Fig. 2). One plasmid carrying a mutation in the protein-coding region was chosen for further analysis. Although *ssc1* cells carrying the temperature-sensitive mutant *ssc1* (BC100(*ssc1-2*)) grew well at 23 °C, after transfer to 37 °C the growth rate rapidly decreased, and growth ceased before one doubling had occurred. The *ssc1-2* mutation is recessive; *ssc1-2/SSC1* heterozygotes have wild-type growth rates at 23 °C and 37 °C (data not shown).

To test whether a defect in *SSC1* results in a defect in import of proteins into mitochondria, protein extracts were prepared from *ssc1* cells carrying the wild-type (BC100(*SSC1*)) or mutant gene (BC100(*ssc1-2*)) (subsequently referred to as 'wild-type' and 'mutant', respectively) and analysed by immunodecoration with antibodies against nuclear-encoded mitochondrial proteins (Fig. 3a). Mutant cells shifted to non-permissive temperature accumulated the precursor forms of hsp60 (Fig. 3a), Ssc1p itself and F₁-ATPase subunit β (F₁ β) (data not shown). Little or no precursor was detected at permissive temperature. As a control, temperature-sensitive *mif1* cells, that are known to be defective in translocation and processing of precursor proteins (M. Y. Cheng and A. L. Horwich, personal communication)^{2,24}, showed a similar accumulation of precursor proteins on shift to non-permissive temperature. As expected, little or no precursor accumulated in cells carrying the wild-type *SSC1* gene at 23 °C or after shift to 37 °C (Fig. 3a).

By pulse-labelling of cells with [³⁵S]methionine, we could determine whether the defect in the import of precursors occurred soon after the shift to non-permissive temperature. BC100(*ssc1-2*) cells and *mif1* cells, radiolabelled 10 min after shift, showed a massive accumulation of precursor proteins of F₁ β , Ssc1p (Fig. 3b) and hsp60 (data not shown). Unlike the *mif1* cells, BC100(*ssc1-2*) cells labelled at 23 °C accumulated a small amount of precursor, indicating a slight defect of protein

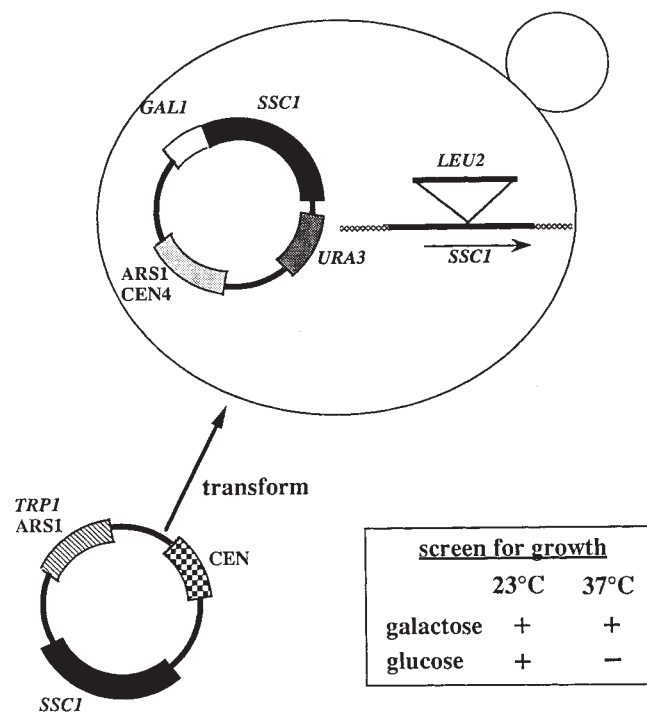


FIG. 2 Screen for temperature-sensitive *ssc1* yeast mutants. Strain BC100(*GAL1::SSC1*)(*MAT* α *leu2-3,112* Δ *trp1* *lys2* *ura3-52* *ssc1-1* *pGAL1::SSC1*(*URA3*)) was used. The *ssc1-1* allele is a disruption mutation constructed by inserting the *LEU2* gene into the *SalI* site in the codon of amino acid 318 in the protein-coding region of *SSC1*. The *GAL1::SSC1* fusion was constructed by ligating plasmid pMB272 (a gift from Mark Johnston) with a 2.3 kilobase (kb) *Bam*HI-*Bgl*II fragment containing the *SSC1* gene with a *Bam*HI linker at position -70 (with +1 being the A of the initiating ATG)¹⁷. A plasmid (pJK808A) carrying a 3.2 kb *Xba*I-*Bgl*II fragment that contained the entire *SSC1* gene with its own promoter in the centromeric vector, pYEcen30 (CEN3, ARS1 and *TRP1*), was treated with 1 M hydroxylamine at 37 °C for 24 h. The plasmid mutagenized *in vitro* was used to transform BC100(*GAL1::SSC1*). Transformants were screened for temperature-sensitive growth at 37 °C on YPD (1% Bacto-yeast extract, 1% Bacto-peptone, 2% dextrose) plates. Plasmids were recovered from the temperature-sensitive transformants and used to transform strain BC100(*GAL1::SSC1*) to confirm that the temperature-sensitive growth on glucose-based media was caused by the plasmid. Segments of the candidate plasmids were re-cloned into unmutagenized pJK808A to determine if the mutation(s) responsible for the ts growth phenotype were in the *SSC1* protein coding region. One of the *SSC1* genes with a mutation(s) within a 1.4-kb *Cla*I fragment (between the codons for amino acids 35-501) was chosen for further analysis and named *ssc1-2*. The *pGAL1*(*SSC1*(*URA3*)) plasmid was cured from strain BC100(*GAL1::SSC1*; *ssc1-2*) to give BC100(*ssc1-2*). Strain BC100(*GAL1::SSC1*) was transformed with unmutagenized pJK808 and then transformants were cured of *pGAL1*(*SSC1*(*URA3*)) to give the control strain BC100(*SSC1*).

import even under permissive growth conditions. As expected, no labelled precursor was found in BC100(*SSC1*) cells at 23 °C or 37 °C. Together these results indicate a defect in import of several mitochondrial precursor proteins in *ssc1*⁻ cells.

To determine the localization of the accumulated precursor in the mutant cells, fractionation experiments were carried out. Almost all the precursor of $F_1\beta$ labelled after shift to non-permissive temperature was present in the mitochondrial fraction (Fig. 3c). Unlike the mature form in wild-type cells, the precursor associated with the mutant mitochondria was accessible to added protease (Fig. 3d). These results suggest that inactivation of Ssc1p leads to a block in mitochondrial protein import.

Ssc1p in protein translocation

The accumulation of precursor proteins *in vivo* suggests that one or more step(s) in the import and assembly pathway of mitochondrial proteins depend(s) on functional Ssc1p. However, as in the analysis of other yeast mutants *in vivo*^{15,24-26}, this finding *per se* does not allow determination of which step actually requires Ssc1p and if the putative role of Ssc1p in protein import is a direct or indirect one.

A detailed characterization of the role of Ssc1p in import of precursor proteins required *in vitro* analysis. For these studies, precursor proteins were synthesized in rabbit reticulocyte lysates in the presence of [³⁵S]methionine. Mitochondria were isolated from *ssc1* cells carrying the wild-type or mutant *SSC1* gene and preincubated for 10 min at 37 °C. Import of the precursors into mitochondria was performed at 25 °C and assessed by the proteolytic processing of the precursors and the protection of the imported proteins against externally added proteinase K^{23,27,28}.

Import of the precursor of cytochrome b_2 into the mutant mitochondria (Fig. 4a). The total amount of precursor in the mitochondria was strongly decreased compared with wild-type import reactions (in the absence of proteinase K) was not reduced after incubation with the mutant mitochondria, excluding unspecific degradation of precursor (see below).

The translocation of precursor proteins across the two mitochondrial membranes can be divided experimentally into two subsequent reactions²⁸⁻³³. First, the precursor protein is inserted into mitochondrial contact sites such that the amino-terminal presequence reaches the matrix space where it is proteolytically cleaved, while a major portion of the precursor is still located on the cytosolic side. Second, the remainder of the precursor is unfolded and also translocated through contact sites, leading to complete translocation of the precursor into the matrix. Only the initial insertion step, but not the completion of translocation, depends on the membrane potential $\Delta\Psi$ across the inner membrane. We used the precursors of $F_1\beta$ and of the Fe/S protein of the bc₁-complex^{29,30,33} to analyse which of the two transport steps was preferentially affected by the mutational alteration of Ssc1p. The precursor proteins were efficiently inserted into the mutant mitochondria and specifically processed by the matrix processing peptidase (Fig. 4b). Complete translocation of the precursors to a protease-protected location, however, was impaired in the mutant mitochondria, indicating accumulation of the precursor proteins in contact sites, thereby spanning the outer and inner membranes. Similarly, a hybrid protein between the presequence of F_0 -ATPase subunit 9 and complete dihydrofolate reductase (Su9-DHFR)^{16,31,34} was accumulated in contact sites of the mutant mitochondria (Fig. 4c). Thus, the $\Delta\Psi$ -independent completion of translocation is defective in the mutant mitochondria. These results exclude the trivial possibility

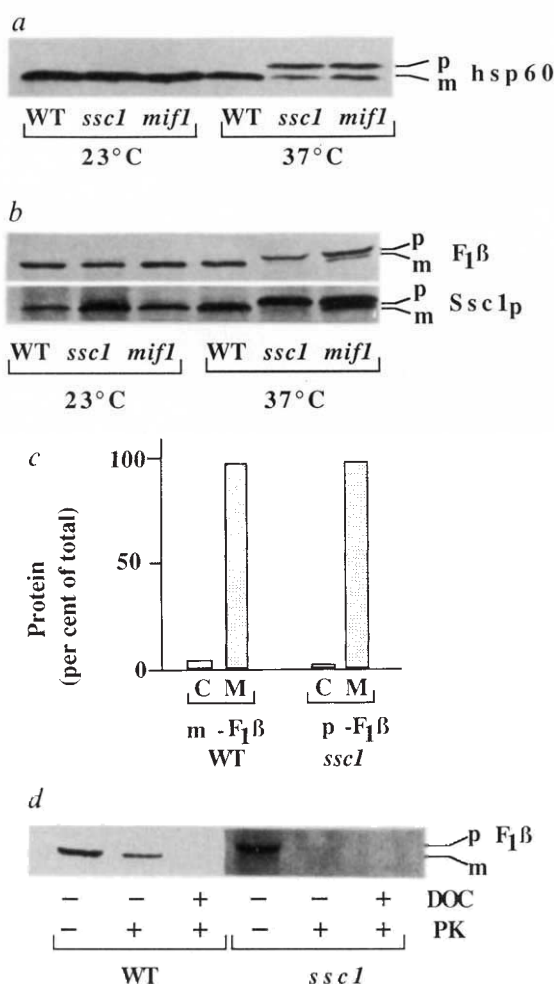
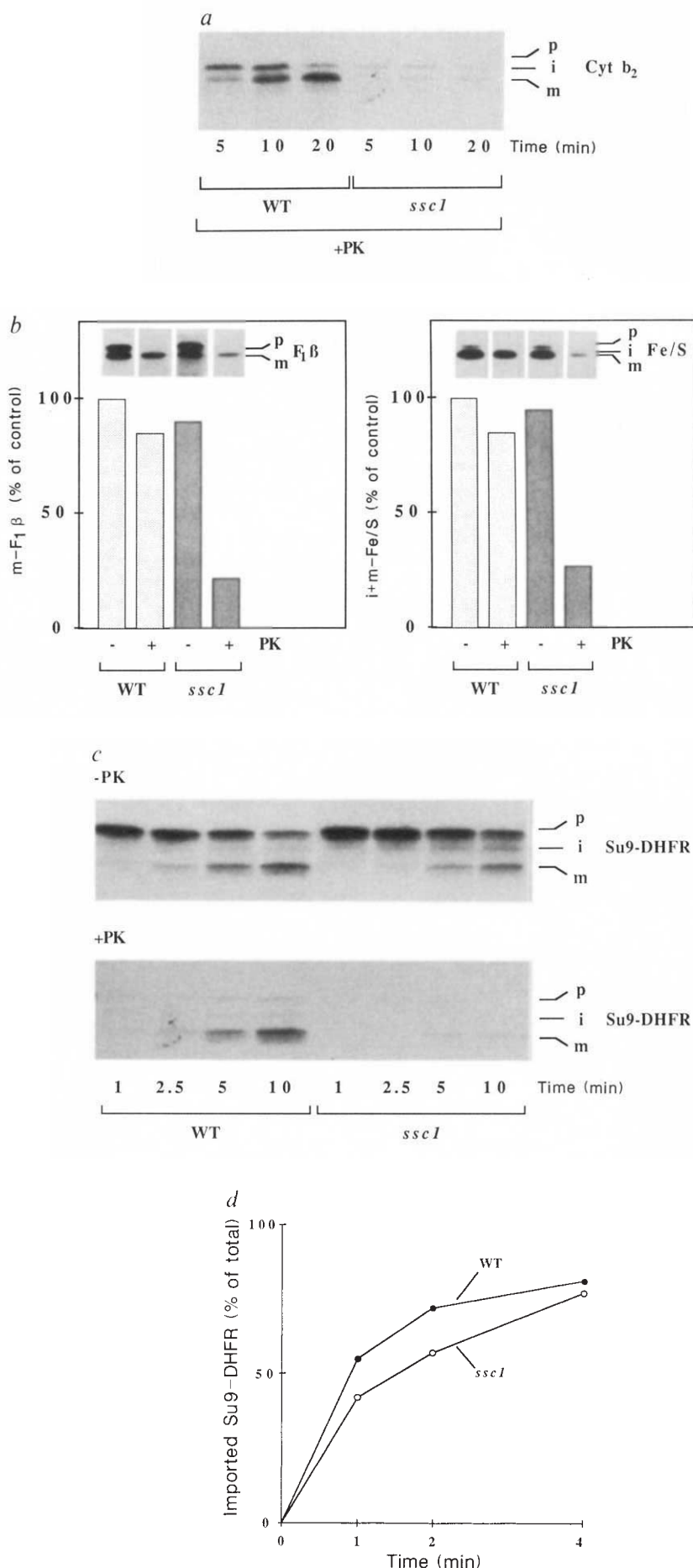


FIG. 3 Accumulation of mitochondrial precursor proteins in yeast cells with defective Ssc1p. **a**, Immunoblot analysis of mitochondrial protein hsp60 in *ssc1* mutant cells. WT, wild-type (BC100(*SSC1*)); *ssc1*, mutant (BC100(*ssc1*-2)). 23 °C, cells grown and maintained at 23 °C; 37 °C, cells grown at 23 °C and shifted to 37 °C before harvest. p, Precursor; m, mature-sized protein. **b**, Pulse labelling of proteins in mutant cells. Immunoprecipitation of radio-labelled $F_1\beta$ and Ssc1p. **c**, Separation of mitochondrial and cytoplasmic fractions. C, cytoplasmic fraction; M, mitochondrial fraction. **d**, Protease-accessibility of accumulated precursor. PK, proteinase K; DOC, deoxycholate. METHODS. **a**, WT (BC100(*SSC1*)), *ssc1* (mutant; BC100(*ssc1*-2)) and *mif1* cells were grown in YPD media at 23 °C. At the early log phase ($OD_{600} \sim 1.0$), the cultures were divided; one aliquot remained at 23 °C, the other was shifted from 23 °C to 37 °C. One hour after temperature-shift, 20 OD_{600} units of cells were harvested and protein extracts were prepared by glass bead lysis⁵⁸. Protein from one OD_{600} unit of cells were loaded on a SDS (7.5%) polyacrylamide gel, electrophoresed, transferred to immobilon membranes (Millipore) and immunoblotted with antisera directed against hsp60. **b**, Cells were grown at 23 °C in selective minimal media (0.67% Bacto-yeast nitrogen base, 2% glucose) lacking methionine. During early log phase (at $OD_{600} \sim 1.0$), 10 OD -units of cells were collected and resuspended in 1 ml of fresh labelling medium. After 10-min preincubation at 23 °C or 37 °C, [³⁵S]methionine (100 μ Ci ml^{-1}) was added. After 10 min, cells were put on ice and NaN_3 and cycloheximide added to 10 mM and 100 μ g ml^{-1} , respectively. Protein extracts were prepared. Aliquots containing $\sim 1 \times 10^6$ c.p.m. were adjusted to 0.5% SDS and immunoprecipitated⁵⁹ using antibodies specific for $F_1\beta$ or Ssc1p and protein A-Sepharose. **c**, Cells were grown as described in **b**. Five OD_{600} units of cells were harvested, resuspended in 2 ml of fresh glucose-based media and shifted to 37 °C. After 30 min, 400 μ Ci [³⁵S]methionine was added and incubation carried out for an additional 30 min. The labelling was terminated by addition of NaN_3 and 20 OD_{600} units of unlabelled cells were added as carrier. After preparation of spheroplasts, mitochondrial and cytoplasmic fractions were prepared^{15,23}. Equivalent amounts of cytoplasmic and mitochondrial fractions were immunoprecipitated using $F_1\beta$ -specific antibodies as described in **b**. **d**, Mitochondrial fractions prepared from cells labelled from 30–60 min after shift to 37 °C as described in **c**, were treated with proteinase K (230 μ g ml^{-1}) in the presence or absence of 0.45% deoxycholate (DOC) for 5 min at 0 °C. Aliquots (0.5 $\times 10^6$ c.p.m., WT; 2 $\times 10^6$ c.p.m., *ssc1*-2) were immunoprecipitated using $F_1\beta$ -specific antibody.

FIG. 4 Deficiency of Ssc1p impairs protein translocation through mitochondrial contact sites. *a*, Kinetics of import of *in vitro*-synthesized cytochrome b_2 into isolated mitochondria. *b*, Accumulation of the precursors of $F_1\beta$ and Fe/S protein in mitochondrial contact sites. The amounts of total processed precursor proteins and of protease-protected processed proteins are compared. *c*, Accumulation of Su9-DHFR in contact sites. Kinetics of processing and transport to a protease-protected location of the fusion protein Su9-DHFR. *d*, Urea-denatured Su9-DHFR is efficiently imported. WT, wild-type mitochondria (BC100(*SSC1*)); *ssc1*, mutant mitochondria (BC100(*ssc1-2*)); PK, proteinase K; p, i, m, precursor-, intermediate- and mature-sized forms of a protein, respectively.

METHODS. Wild-type (BC100(*SSC1*)) and *ssc1-2* cells were grown on YPG medium for 20 h at 23 °C. Exponentially growing cells were collected and mitochondria were isolated as described²³. Precursor proteins were synthesized in rabbit reticulocyte lysates by coupled transcription/translation and labelled with [³⁵S]methionine²⁷. Mitochondria (0.25 mg protein ml⁻¹) were preincubated for 10 min at 37 °C and 3 min at 25 °C in import buffer (3% BSA, 220 mM sucrose, 70 mM KCl, 2.5 mM MgCl₂, 10 mM MOPS, pH 7.2). Reticulocyte lysate (10% (v/v)) containing the indicated radiolabelled precursor proteins, NADH (2 mM) and ATP (1 mM) were added and the import reaction was incubated at 25 °C. *a*, Aliquots (containing precursor of cytochrome b_2) were withdrawn at the indicated time, diluted threefold in ice-cold SEM buffer (250 mM sucrose, 1 mM EDTA, 10 mM MOPS, pH 7.2) and incubated with proteinase K (20 µg ml⁻¹) for 15 min at 0 °C. After addition of 1 mM PMSF, mitochondria were reisolated by centrifugation and analysed by SDS-PAGE and fluorography. *b*, Aliquots (containing precursors of $F_1\beta$ and Fe/S protein) were withdrawn after 20 min and incubated at 0 °C in the presence or absence of proteinase K. Mitochondria were reisolated and analysed as described above. The fluorographs (inserts) were quantified by laser densitometry (columns). The amount of processed protein in wild-type mitochondria was taken as 100%. *c*, Aliquots (containing precursor of Su9-DHFR) were withdrawn at the indicated times and further treated as described above. *d*, Reticulocyte lysate containing Su9-DHFR was precipitated with ammonium sulphate at 66% saturation and dissolved in 8 M urea, 20 mM Tris, pH 7.5 (ref. 16). The urea-denatured precursor was diluted 15-fold into the import reaction. After incubation for the indicated times at 25 °C, aliquots were treated with proteinase K. The mitochondria were reisolated and analysed as described above. The amount of imported protein is expressed as the percentage of total added precursor protein. The mutant mitochondria contained 98–104% of the wild-type levels of marker proteins, cytochrome *c*, cytochrome b_2 and fumarase, under the various import conditions (presence and absence of proteinase K), confirming the intactness of mitochondrial outer and inner membranes as described^{23,33}.



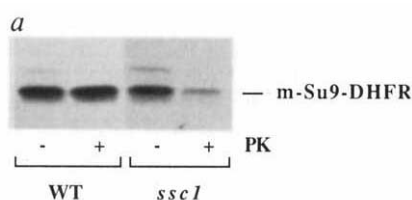
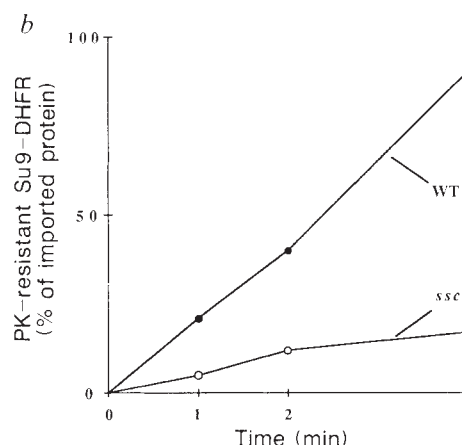


FIG. 5 Accumulation of unfolded precursor protein in the mutant mitochondria. Urea-denatured Su9-DHFR was imported into isolated wild-type (WT) and *ssc1-2* mitochondria. The amount of protease-resistant (folded) protein on lysis of the mitochondrial membranes with detergent is compared. PK, proteinase K.

METHODS. Import of urea-denatured Su9-DHFR for 4 min (a) or the indicated times (b) and incubation of the mitochondria with proteinase K was performed as described in the legend to Fig. 4 (ref. 16). Mitochondria were reisolated, resuspended in SEM buffer containing 0.4% digitonin and divided into halves. One half (+PK) received proteinase K ($10 \mu\text{g ml}^{-1}$) and was incubated for 10 min on ice. Trichloroacetic acid (10%) was added to each aliquot and the precipitated proteins were analysed by SDS-PAGE, fluorography and laser-densitometry. b, The amount of protease-resistant protein



(treatment with proteinase K on lysis of mitochondria with digitonin) is given as percentage of total imported protein (protected against proteinase K in intact mitochondria) at each time point.

that the translocation defect might be due to a diminished membrane potential.

As the completion of membrane translocation includes the unfolding of large parts of the precursors on the cytosolic side, we reasoned that an artificial unfolding of precursors should reduce the translocation defect into the mutant mitochondria. The hybrid protein Su9-DHFR was thought to be a suitable substrate for an analysis of this problem as we had previously found that Su9-DHFR could be transported into mitochondria after denaturation by a preincubation in 8 M urea and subsequent dilution into the import reaction¹⁶. Su9-DHFR denatured with urea was efficiently imported into the mutant mitochondria at rates that were close to the import rates observed with wild-type mitochondria (Fig. 4d), in contrast to the results obtained with the partially folded Su9-DHFR synthesized in reticulocyte lysate (Fig. 4c). This difference suggests an involvement of Ssc1p in unfolding of the precursor and confirms that the transport defect is not related to the $\Delta\Psi$ -dependent step. Moreover, it excludes the possibility that the decreased amounts of protease-protected proteins observed in mutant mitochondria (Fig. 4a-c) are caused by an increased susceptibility of mutant mitochondria to proteolytic attack. The intactness of the mitochondria was also confirmed by the analysis of several marker proteins (see Fig. 4 legend). We conclude that Ssc1p is required for the completion of transport of precursor proteins through mitochondrial contact sites, including the unfolding of a portion of the precursor on the cytosolic side. The accumulation of unprocessed precursors *in vivo* (Fig. 3) is probably due to saturation of import sites by the massive amounts of arrested precursors, thereby leading to a 'back-up' block at an earlier import stage.

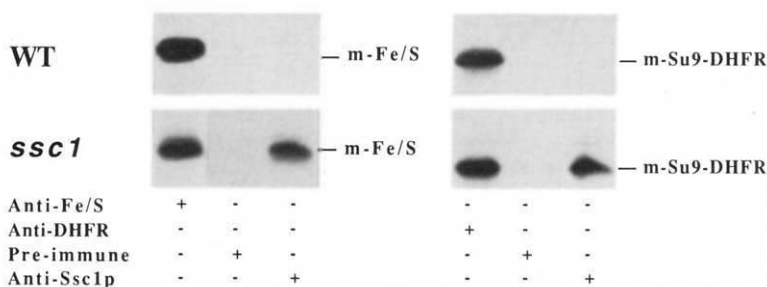
Ssc1p in (re)folding of imported proteins

As artificial denaturation of precursors allowed a circumvention of the translocation defect, it was possible to investigate if further import steps occurring in the matrix would be affected in the mutant mitochondria. Of particular importance is the refolding of imported polypeptide chains in the matrix, which was previously shown to involve mitochondrial hsp60 (ref. 16). For the experimental analysis, the hybrid protein Su9-DHFR was used as the folding state of its DHFR domain can be investigated by assaying its sensitivity towards proteolytic attack. After import of Su9-DHFR into mitochondria and removal of its presequence by the processing peptidase, the mitochondria were lysed with detergent and treated with proteinase K: the unfolded protein was sensitive to proteolytic digestion whereas the folded polypeptide (essentially consisting of mature DHFR) exhibited a high protease resistance¹⁶. In the experiment described in Fig. 5, we found that Su9-DHFR was highly protease-sensitive after import into the mutant mitochondria, indicating that it did not achieve the tightly folded conformation. In wild-type mitochondria, the protein became folded and thus protease-resistant (Fig. 5). We conclude that the (re)folding of proteins in the mitochondrial matrix requires functional Ssc1p.

Ssc1p could be directly involved in translocation and refolding of precursor proteins by a physical interaction with the precursors. Alternatively, the defects observed in the mutant mitochondria could be indirect, because of an impairment of components of the import machinery by mutated Ssc1p. A direct interaction of precursor proteins with Ssc1p would demonstrate the first, more interesting possibility. As such a complex would be a transient intermediate, the reaction could be demonstrated only by selectively blocking release of precursors from Ssc1p. To see

FIG. 6 Association of precursor proteins with Ssc1p. Fe/S protein and urea-denatured Su9-DHFR imported into mutant mitochondria (*ssc1*) are co-precipitated with antibodies specific for Ssc1p on lysis of mitochondria with detergent. WT, wild-type mitochondria.

METHODS. Fe/S protein and urea-denatured Su9-DHFR were imported into isolated wild-type (WT) and *ssc1-2* (*ssc1*) mitochondria as described in the legend of Fig. 4. Immunoglobulin G from 15 μl rabbit antisera specific for Fe/S protein, DHFR or Ssc1p, or pre-immune serum were pre-bound to protein A-Sepharose. Mitochondria (15 μg of protein per sample) were dissolved in 0.1% Triton X-100, 100 mM NaCl, 10 mM Tris, pH 7.5 (150 μl) and incubated with the protein A-Sepharose under gentle mixing for 1 h at 4°C. The immunoprecipitates were



washed twice in the buffer described above and once in 10 mM Tris, pH 7.5, dissolved in SDS-containing buffer and analysed by SDS-PAGE and fluorography.

if the mutation of Ssc1p led to impairment of a step(s) required for release of precursors without abolishing the initial interaction of precursors with Ssc1p, we tried to purify a complex between precursor proteins and mutant Ssc1p. Using the antibodies specifically directed against the carboxyl-terminus of Ssc1p, we coprecipitated imported Fe/S protein and Su9-DHFR after lysis of mitochondria with detergent (Fig. 6). This co-immunoprecipitation provides strong support for a physical interaction of the precursors with Ssc1p and suggests a direct involvement of Ssc1p in mitochondrial protein import. In our control experiment, Fe/S protein and Su9-DHFR in wild-type mitochondria were fully translocated and folded^{16,30,34}, and thus not co-precipitated with antibodies against Ssc1p (Fig. 6).

Discussion

Hsp70 (Ssc1p) in the mitochondrial matrix is required for import of precursor proteins into mitochondria. Specifically, completion of protein transport through contact sites, including unfolding and membrane translocation of the main parts of the precursor proteins, and refolding of imported proteins in the matrix, depend on functional Ssc1p. The isolation of a complex between precursor proteins and Ssc1p suggests a direct interaction of Ssc1p with precursor proteins.

We propose that Ssc1p mediates between the translocation apparatus in contact sites and the folding machinery in the matrix of mitochondria. Ssc1p would bind the precursor protein as it emerges on the matrix side of contact sites and thereby support the completion of translocation, including facilitation of unfolding of the polypeptide chain by the import apparatus on the cytosolic side. By interaction with one or more Ssc1p molecule(s) the precursor would be 'pulled' towards the inside of mitochondria, perhaps thereby facilitating the unfolding reaction. In addition, tight binding of the precursor in transit to Ssc1p's could render the translocation vectorial and thus assist the completion of membrane transport. The energy requirement for complete unfolding of many proteins was found to be as low as 5–10 kcal mol⁻¹ (ref. 35). Therefore, it is conceivable that the free energy that might be released by binding of Ssc1p molecules to a polypeptide chain appearing on the matrix side may be considerably larger than the energy required for unfolding of the same polypeptide chain. The exact molecular mechanism of the role of Ssc1p will remain elusive until other components of the unfolding and translocation apparatus of contact sites have been identified. Second, Ssc1p seems to be an initial component in the chain of reactions leading to refolding of imported proteins. We previously reported that the refolding reaction *per se* could occur in association with hsp60 in the matrix¹⁶. Ssc1p could directly participate in this refolding process. Now, as Ssc1p seems to be a binding protein for precursors in transit through contact sites, we prefer a model in which Ssc1p accepts the incoming precursors, keeps them in an unfolded conformation and possibly mediates their transfer to hsp60. The properties of Ssc1p, as revealed by analysis of the yeast mutant, group it into the class of proteins collectively termed 'molecular chaperones'^{14,36,37} or 'polypeptide chain binding proteins'³⁸, that mediate the folding and assembly of other polypeptides.

The completion of protein transfer through mitochondrial contact sites does not depend on the addition of an external energy source (when one round of translocation is analysed)³⁹. The binding of precursor proteins to mitochondrial hsp70 and possibly also hsp60 could thus provide the energy for membrane translocation. Hydrolysis of ATP might then be required for releasing the precursor proteins from the chaperones, setting the chaperones free for new rounds of transport.

As the previously described hsp70s (cytosolic hsp70s, BiP in the ER, the DnaK protein in *E. coli* and probably also chloroplast hsp70s^{22,40}) are very similar to Ssc1p^{17,19,41–43}, it is tempting to speculate that they perform functions similar to those of mitochondrial hsp70. The proposed role of cytosolic hsp70s in

maintaining precursor proteins in an unfolded conformation, supporting the translocation of proteins into organelle membranes^{6–10,44,45}, agrees with the indicated functions of Ssc1p. BiP has been strongly implicated in the assembly of protein complexes in the lumen of the ER^{9,37,43,46–51}. BiP could be involved in protein translocation⁵²; but such a role has been questioned^{51,53}. The results reported here would support the model that BiP may be required at early stages, for translocation of proteins into the ER and for the reactions leading to refolding of imported proteins. Such a function of hsp70s is consistent with the finding⁵⁴ that both cytosolic hsp70s and BiP can interact with peptides that might resemble structures of loosely folded precursor proteins and agrees with the proposal^{38,45} that polypeptide chains emerging on the surface of ribosomes or the non-cytosolic surface of organelle membranes will interact productively with hsp70s. Phillips and Silhavy⁵⁵ found that DnaK, when overexpressed, stimulates protein export in *E. coli*. Biochemical studies on a role of DnaK in modulating the conformation of precursor proteins seem to be promising, particularly as Ssc1p is more similar to DnaK than it is to any eukaryotic hsp70 (ref. 17). As the mechanisms of mitochondrial and chloroplast protein uptake are rather similar^{56,57}, chloroplast hsp70s^{22,40}, which have not yet been functionally characterized, could perform a function equivalent to that of mitochondrial hsp70. Analysis of the function of Ssc1p by using translocation intermediates of mitochondrial precursor proteins thus has implications for a number of systems involved in the transport, folding and assembly of proteins. □

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- Attardi, G. & Schatz, G. A. *Rev. Cell Biol.* **4**, 289–333 (1988).
- Hartl, F.-U. & Neupert, W. *Science* **247**, 930–938 (1990).
- Pfanner, N. & Neupert, W. A. *Rev. Biochem.* **59**, 331–353 (1990).
- Lindquist, S. & Craig, E. A. *Rev. Genet.* **22**, 631–677 (1988).
- Werner-Washburne, M., Stone, D. E. & Craig, E. A. *Molec. cell. Biol.* **7**, 2568–2577 (1987).
- Deshales, R. J., Koch, B. D., Werner-Washburne, M., Craig, E. A. & Schekman, R. *Nature* **332**, 800–805 (1988).
- Murakami, H., Pain, D. & Blobel, G. *J. Cell Biol.* **107**, 2051–2057 (1988).
- Chirico, W. J., Waters, M. G. & Blobel, G. *Nature* **332**, 805–810 (1988).
- Pelham, H. *Nature* **332**, 776–777 (1988).
- Zimmermann, R., Sagstetter, M., Lewis, M. J. & Pelham, H. R. B. *EMBO J.* **7**, 2875–2880 (1988).
- Hendrix, R. W. *J. molec. Biol.* **129**, 375–392 (1979).
- Hemmingsen, S. M. et al. *Nature* **333**, 330–334 (1988).
- Reading, D. S., Hallberg, R. L. & Myers, A. M. *Nature* **337**, 655–659 (1989).
- Ellis, R. J. & Hemmingsen, S. M. *Trends biochem. Sci.* **14**, 339–342 (1989).
- Cheng, M. Y. et al. *Nature* **337**, 620–625 (1989).
- Ostermann, J., Horwich, A. L., Neupert, W. & Hartl, F.-U. *Nature* **341**, 125–130 (1989).
- Craig, E. A. et al. *Molec. cell. Biol.* **9**, 3000–3008 (1989).
- Craig, E. A., Kramer, J. & Kosic-Smithers, J. *Proc. natn. Acad. Sci. U.S.A.* **84**, 4156–4160 (1987).
- Engman, D. M., Kirchhoff, L. V. & Donelson, J. E. *Molec. cell. Biol.* **9**, 5163–5168 (1989).
- Leustek, T., Dalie, B., Amir-Shapira, D., Brot, N. & Weissbach, H. *Proc. natn. Acad. Sci. U.S.A.* **86**, 7805–7808 (1989).
- Mizzen, L. A., Chang, C., Garrels, J. I. & Welch, W. J. *J. biol. Chem.* **264**, 20664–20675 (1989).
- Amir-Shapira, D., Leustek, T., Dalie, B., Weissbach, H. & Brot, N. *Proc. natn. Acad. Sci. U.S.A.* **87**, 1749–1752 (1990).
- Hartl, F.-U., Ostermann, J., Guirad, B. & Neupert, W. *Cell* **51**, 1027–1037 (1987).
- Pollock, R. A. et al. *EMBO J.* **7**, 3493–3500 (1988).
- Yaffe, M. P., Ohta, S. & Schatz, G. *EMBO J.* **4**, 2069–2074 (1985).
- Hartl, F.-U., Pfanner, N., Nicholson, D. & Neupert, W. *Biochim. biophys. Acta* **988**, 1–45 (1989).
- Pfanner, R., Steger, H. F., Rassow, J., Pfanner, N. & Neupert, W. *J. Cell. Biol.* **107**, 2483–2490 (1988).
- Rassow, J. et al. *J. Cell Biol.* **109**, 1421–1428 (1989).
- Schleyer, M. & Neupert, W. *Cell* **43**, 339–350 (1985).
- Hartl, F.-U., Schmidt, B., Wachter, E., Weiss, H. & Neupert, W. *Cell* **47**, 939–951 (1986).
- Pfanner, N., Tropschug, M. & Neupert, W. *Cell* **49**, 815–823 (1987).
- Pfanner, N., Hartl, F.-U., Guirad, B. & Neupert, W. *Eur. J. Biochem.* **169**, 289–293 (1987).
- Schwaiger, M., Herzog, V. & Neupert, W. *J. Cell Biol.* **105**, 235–246 (1987).
- Pfanner, N., Müller, H. K., Harmey, M. A. & Neupert, W. *EMBO J.* **6**, 3449–3454 (1987).
- Pace, C. N. *Trends biochem. Sci.* **15**, 14–17 (1990).
- Laskey, R. A., Honda, B. M., Mills, A. D. & Finch, J. T. *Nature* **275**, 416–420 (1978).
- Ellis, J. *Nature* **328**, 378–379 (1987).
- Rothman, J. E. *Cell* **59**, 591–601 (1989).
- Pfanner, N. et al. *J. biol. Chem.* **265**, in the press (1990).
- Marshall, J. S., DeRocher, A. E., Keegstra, K. & Vierling, E. *Proc. natn. Acad. Sci. U.S.A.* **87**, 374–378 (1990).
- Rose, M. D., Misra, L. M. & Vogel, J. P. *Cell* **57**, 1211–1221 (1989).
- Normington, K., Kohno, K., Kozutsumi, Y., Gething, M.-J. & Sambrook, J. *Cell* **57**, 1223–1236 (1989).
- Munro, S. & Pelham, H. R. B. *Cell* **46**, 291–300 (1986).
- Waegemann, K., Paulsen, H. & Soli, J. *FEBS Lett.* **261**, 89–92 (1990).
- Beckmann, R. P., Mizzen, L. A. & Welch, W. J. *Science* **248**, 850–854 (1990).
- Bole, D. G., Hendershot, L. M. & Kearney, J. F. *J. Cell Biol.* **102**, 1558–1566 (1986).
- Copeland, C. S., Doms, R. W., Bolzau, E. M., Webster, R. G. & Helenius, A. *J. Cell Biol.* **103**, 1179–1191 (1986).

48. Gething, M.-J., McCammon, K. & Sambrook, J. *Cell* **46**, 939-950 (1986).
 49. Pelham, H. R. B. *Cell* **46**, 959-961 (1986).
 50. Hendershot, L., Bole, D., Köhler, G. & Kearney, J. F. *J. Cell Biol.* **104**, 761-767 (1987).
 51. Kassenbrock, C. K., Garcia, P. D., Walter, P. & Kelly, R. B. *Nature* **333**, 90-93 (1988).
 52. Vogel, J. P., Misra, L. M. & Rose, M. D. *J. Cell Biol.* **110**, 1885-1895 (1990).
 53. Nicchitta, C. V. & Blobel, G. *Cell* **60**, 259-269 (1990).
 54. Flynn, G. C., Chappell, T. G. & Rothman, J. E. *Science* **245**, 385-390 (1989).
 55. Phillips, G. J. & Silhavy, T. J. *Nature* **344**, 882-884 (1990).
 56. Pfanner, N., Hartl, F.-U. & Neupert, W. *Eur. J. Biochem.* **175**, 205-212 (1988).
 57. Keegstra, K. *Cell* **56**, 247-253 (1989).

58. Nicolet, C. M. & Craig, E. A. *Molec. cell. Biol.* **9**, 3638-3646 (1989).
 59. Kessler, S. W. *Meth. Enzym.* **73**, 442-459 (1981).

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LETTERS TO NATURE

The potential distribution around growing fractal clusters

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THE process of diffusion-limited aggregation (DLA) is a common means by which clusters grow from their constituent particles, as exemplified by the formation of soot and the aggregation of colloids in solution. DLA growth is a probabilistic process which results in the formation of fractal (self-similar) clusters. It is controlled by the harmonic measure (the gradient of the electrostatic potential) around the cluster's boundary. Here we show that interactive computer graphics can provide new insight into this potential distribution. We find that points of highest and lowest growth probability can lie unexpectedly close together, and that the lowest growth probabilities may lie very far from the initial seed. Our illustrations also reveal the prevalence of 'fjords' in which the pattern of equipotential lines involves a 'mainstream' with almost parallel walls. We suggest that an understanding of the low values of the harmonic measure will provide new understanding of the growth mechanism itself.

The DLA model of Witten and Sander¹ captures the essential fractal aspects of a wide range of physical phenomena²⁻⁷, such as particle aggregation, dielectric breakdown, viscous fingering and electrochemical deposition. In DLA growth, an 'atom' executes Brownian motion until it hits, and becomes attached to, the curve that bounds a given 'target' or 'seed'. Another atom is then launched, and the whole process repeats. Initially, the growth rule and the pattern are very simple, but both become extremely complex in time. The distribution of hitting points of the atoms on the boundary of a fixed pattern becomes increasingly irregular and complex with repeated application of the rules. Simultaneously, the pattern becomes more complex (Fig. 1). Overwhelming evidence from computer simulations¹⁻⁷ indi-

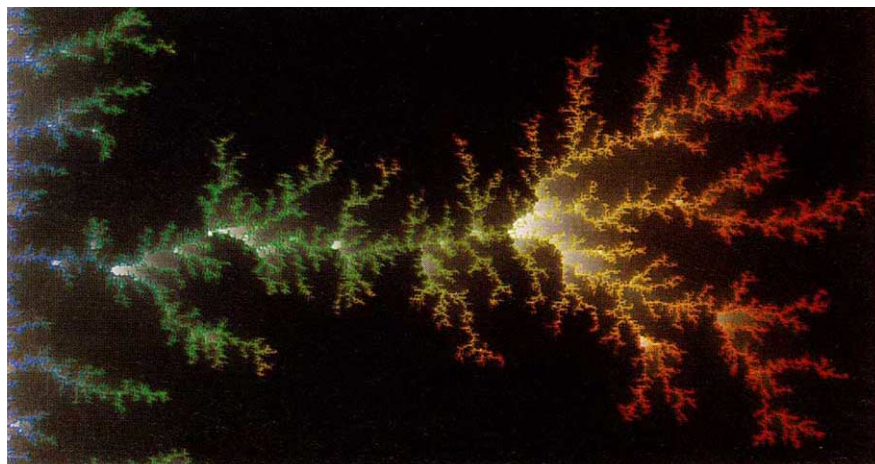
cates that these patterns are near self-similar fractals⁸, meaning that their complication is about the same at all scales of observation that are sufficiently above the scale of the atom.

The distribution of the hitting points on the target's boundary is the harmonic measure, μ (ref. 9). This measure is obtained by solving the Laplace equation for the (electrostatic) potential, the boundary of the cluster being put at zero potential and a 'circle at infinity' being put at unit potential. The gradient of the potential at a point P of the boundary defines the harmonic measure $\mu(P)$ at P . It is approximated by the potential at the lattice point nearest to P (ref. 10), and is normalized to add up to one. The study of the laplacian potentials and their harmonic measures has been enriched by the example of DLA, because the process creates its own boundary conditions. The difficulties encountered in analytical approaches have made DLA a challenge in theoretical physics^{6,7,11-14} and in pure mathematics.

Figures 1, 2 and 3 show half of one of the many clusters we have grown on a cylindrical lattice, the initial target being the cylinder base. The many clusters drawn on a lattice in the original circle geometry^{1,10}, in which the initial target is a cell in a square lattice, look similar (see cover figure). The cylindrical cluster shown in Figs 1-3 was grown to a height equal to its base $L=512$, and contains $\sim 30,000$ atoms. Once the cluster is obtained, the custom has been to solve the discrete Laplace equation¹⁰ on the original lattice on which DLA has been grown. But in the resulting illustrations, the potential cannot be seen with sufficient resolution in the many narrow 'fjords'. We therefore solved the Laplace equation on a lattice that was twice as fine. This equation was solved iteratively and the relative computation error was ~ 0.01 .

Figure 1 and the cover figure illustrate our first finding. As is usual in this field⁴, each atom is coloured to indicate the time it joined the cluster. In the background, rendered in black, the potential is above a certain threshold. Elsewhere, the 'whiteness' of the background increases with $-\log(\text{potential})$. The low-potential zones look like ghostly 'cobwebs' extending to spatial scales larger than the atoms. Many occur in the bottom of fjords that may be short, but are narrow. Their location is very sample-dependent, and in many clusters they appear

FIG. 1 'Cobwebs' of very low potentials near a cylindrical cluster of diffusion-limited aggregation. The vertical cylinder base is to the left.



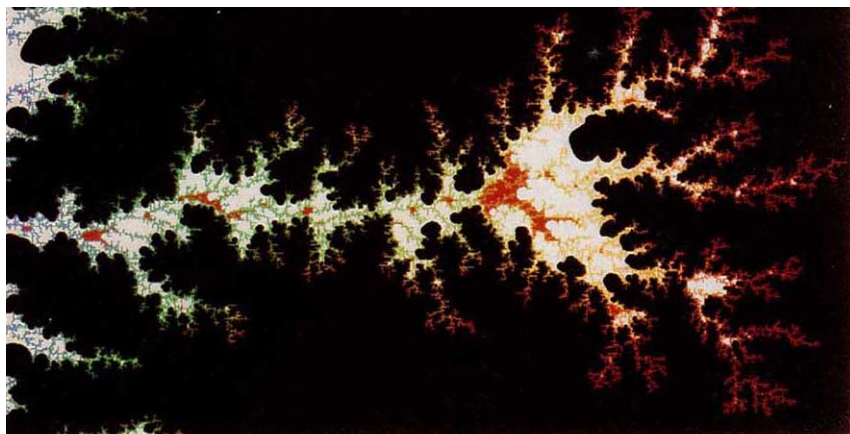


FIG. 2 Low-potential regions around part of a cluster. The smallest value of the growth probability occurs at the lowest point of the largest red domain.



FIG. 3 Equipotential lines around cluster. Each combination of one black and one white zebra stripe marks a fall in the potential by a factor of ten.

between young growing branches positioned near the top of very large trees. The very bright and localized spots seen in Fig. 1 seem irrelevant to the understanding of the structure of DLA above the atomic level, and our conclusions do not depend on their presence or absence.

Figure 2 is another representation of the data in Fig. 1. The smallest potentials from 10^{-25} to 10^{-17} are in red, and the potentials between 10^{-10} and 10^{-17} are in white. The domains of lowest potential are conspicuously absent near the base of the tree.

The rendering used in Fig. 3 is borrowed from the 'zebra stripe' illustrations of the potentials around Julia or Mandelbrot sets¹⁵. Here, the logarithm of the potential is evaluated in base $b = \sqrt{10}$, and odd and even values of the integer part of $-\log_b(\text{potential})$ are plotted in black and white. Given two points not too close to the boundary, one measures the change in $-\log_{10}(\text{potential})$ by counting 'doublets' made of one black and one white stripe.

There is a stark difference in the structure of the trees formed by the fjords and those formed by the clusters. The most striking feature is the abundance of fjords through which the potential field has near constant width, despite the fractal shape of the walls. A stack of near uniformly spaced binary stripes provides a smoothed inner approximation of such a fjord. Rectangular fjords with parallel walls have been considered (for example, in ref. 16), but only because they are easy to treat analytically. Our figures suggest that one could almost characterize DLA clusters as branching just enough to ensure fjords in which the potential exhibits a 'mainstream' with nearly parallel walls, including fjords that are narrow and surprisingly long. Our observations based on zebra striping fit nicely within the mathe-

matical advances of Carleson and Jones¹⁷ concerning Laplacian potentials around fractal boundaries.

Although the location of the smallest growth probabilities in DLA does not seem to have been discussed explicitly in the literature, in our experience there is a widespread expectation that the lowest potentials should be concentrated near the initial seed. It is well known, and follows from a theorem by Carleson and Jones¹⁷, that if DLA were exactly self similar, the harmonic measure would be smallest at the bottoms of the deepest fjords. The effects we report therefore express deviations from exact self-similarity. They do not originate from branching hierarchies, but from the ubiquitous geometric fluctuations, which result in narrow fjords when branches fail to fan out and instead run in parallel or tend to close in on each other.

The reasoning behind the expectation concerning the smallest probabilities is that the initial seed becomes increasingly 'screened' as the cluster grows: at each new stage of screening, the harmonic measure μ is multiplied by some random factor < 1 , adding some positive term to $-\log \mu$. This initial step is the very basis of the original multiplicative multifractals¹⁸. Because the points near the initial seed are subject to the most stages of screening, it seems natural to expect them to correspond in all cases to the largest values of $-\log \mu$. But this assumes that the increment of $-\log \mu$ contributed by each stage of screening has a finite expectation, which is not a safe assumption. On the contrary, our observation that a few stages of screening can be as effective as many (because there are minima in short fjords), combined with extensive numerical work to be reported elsewhere, points toward an infinite expectation value for the increments of $-\log \mu$ in models of DLA. It follows^{16,19-21} that the so called 'thermodynamic' distribution function $f(\alpha)$ is

left-sided. Here α is the Holder exponent, $\alpha = -\log \mu / \log L$. We therefore believe that left-sidedness is not a pathology related to 'old wood' near the initial seed, but a genuine aspect of fractal growth in DLA and hence physically significant. 'Thermodynamic' implies that $f(\alpha)$ is an asymptotic concept. When $f(\alpha)$ is left-sided, however, the convergence to this asymptote is not only slow but 'singular'^{19,21}, meaning that even if a cluster is very large, its 'finite size' $f(\alpha)$ differs in shape from the asymptote. This issue will be discussed elsewhere.

Much of the research in fractal geometry has been done, and the results discussed, using graphical representations⁸. The ability to graph clusters resulting from DLA and related processes has been crucial to the progress in this field⁴. Nevertheless, we believe that this path deserves to be followed much farther. Potentials have been plotted before (refs 22–25 and P. Meakin, unpublished work) but the plots were, in those cases, intended solely to illustrate already known facts.

Here we have discussed only the observations from a careful interactive study of the graphics, and some implications for the theoretical understanding of DLA. Our quantitative analysis of DLA will be reported elsewhere. Combining the potential field and the cluster has emphasized the interplay between the two, revealing new properties, and, we feel, will lead to a better understanding of fractal growth.

Note added in proof: We have now done the experiments using 'off-lattice' aggregates. The results confirm those reported here. □

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- Witten T. A. & Sander, L. M. *Phys. Rev. Lett.* **47**, 1400–1403 (1981).
- Pietronero, L. & Tosatti, E. (eds) *Fractals in Physics* (North-Holland, Amsterdam, 1986).
- Feder, J. *Fractals* (Plenum, New York, 1988).
- Meakin, P. in *Phase Transitions and Critical Phenomena* (eds Domb, C. & Lebowitz, J.) Vol. 12, 355–489 (Academic, New York, 1988).
- Vicsek, T. *Fractal Growth Phenomena* (World Scientific, Singapore, 1989).
- Aharony, A. & Feder, J. eds *Fractals in Physics* (North-Holland, Amsterdam, 1990).
- Aharony, A. & Feder, J. *Physica D* **38**, 1–398 (1989).
- Mandelbrot, B. B. *Fractal Geometry of Nature* (Freeman, New York, 1982).
- Kakutani, S. *Proc. Imper. Acad. Sci. (Tokyo)* **20**, 706–714 (1944).
- Niemeyer, L., Pietronero, L. & Wiesmann, H. J. *Phys. Rev. Lett.* **52**, 1033–1036 (1984).
- Pietronero, L., Erzan, A. & Evertsz, C. J. G. *Phys. Rev. Lett.* **61**, 861–864 (1988).
- Pietronero, L., Erzan, A. & Evertsz, C. S. G. *Physica A* **151**, 207–245 (1988).
- Tsallis, C. (ed.) *Statistical Physics* (North-Holland, Amsterdam, 1990).
- Tsallis, C. *Physica A* **163**, 1–428 (1990).
- Peitgen, H. O. & Richter, P. H. *The Beauty of Fractals* (Springer-Verlag, New York, 1986).
- Blumenfeld, R. & Aharony, A. *Phys. Rev. Lett.* **62**, 2977–2980 (1989).
- Carleson, L. & Jones, P. W. *Duke math. J.* (in the press).
- Mandelbrot, B. B. *J. Fluid Mech.* **62**, 331–358 (1974).
- Mandelbrot, B. B. *Physica A* **168**, 95–111 (1990).
- Lee, J. & Stanley, H. E. *Phys. Rev. Lett.* **61**, 2945–2948 (1988).
- Mandelbrot, B. B., Evertsz, C. J. G. & Hayakawa, Y. *Phys. Rev. A* **42**, 4528–4536 (1990).
- Pietronero, L., Evertsz, C. J. G. & Siebesma, A. P. in *Stochastic Processes in Physics and Engineering* (ed. Albeverio, S. et al.) (Reidel, Dordrecht, 1988).
- Sander, L. M. *Sci. Am.* **256**(1), 82–88 (1987).
- Family, F. & Vicsek, T. *Computers in Physics*, **4**, 44–49 (1990).
- Evertsz, C. J. G., Siebesma, A. P. & Oeindk, F. *Laplacian Fractal Growth*, video (Solid State Physics Laboratory, University of Groningen, 1988).

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Iodine intercalation of a high-temperature superconducting oxide

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INTERCALATION compounds are formed by inserting guest atomic or molecular species between weakly bound (usually by van der Waals forces) slabs of host materials without changing the inner crystal structure of the individual slabs. The best-known

examples of host materials are graphite and the transition metal dichalcogenides¹. These compounds can be made with different stage index n , where n denotes the number of slabs between adjacent intercalated layers. Intercalation provides a unique, well controlled approach to changing the physical and electronic properties of host materials over a wide range¹. If intercalation can be adopted in the layered high-transition-temperature (high- T_c) superconductors, it could lead to the ability to engineer their properties, with a view to investigating the mechanism responsible for high- T_c superconductivity, improving the superconducting properties of the pristine materials, and developing new high- T_c superconductors and superconducting devices. We report here the successful synthesis and preliminary physical characterization of a stage-1 iodine-intercalated high- T_c superconductor, $\text{IBi}_2\text{Sr}_2\text{CaCu}_2\text{O}_y$.

Intercalation is possible only when the binding between the slabs of the host material is very weak. Several of the high- T_c oxide superconductors fall into this class. For example, $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_8$ crystals have a micaceous structure with anisotropic binding, allowing the crystals to be easily cleaved between the double [Bi-O] layers². The interplane bond length between the double [Bi-O] layers is 3.7 Å, whereas the intraplane Bi—O bond length is 2.3 Å (ref. 3).

We have intercalated iodine between the [Bi-O] planes of $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_8$, yielding a new stage-1 compound in which the c axis is increased by 23% over the pristine unit-cell dimension. The stoichiometry of the saturated material is $\text{IBi}_2\text{Sr}_2\text{CaCu}_2\text{O}_y$; it is a bulk superconductor with transition temperature of 80 K.

To prepare the samples, iodine and high-quality single crystals of $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_8$ (with transition temperatures ranging from 82 to 90 K) were sealed in pyrex tubes under a vacuum of $<10^{-3}$ torr and placed in uniform-temperature furnaces. For temperatures less than 100 °C and times shorter than six days, the resulting compounds were not single-phase but a mixture of stage-1 and pristine materials. For temperatures over 250 °C,

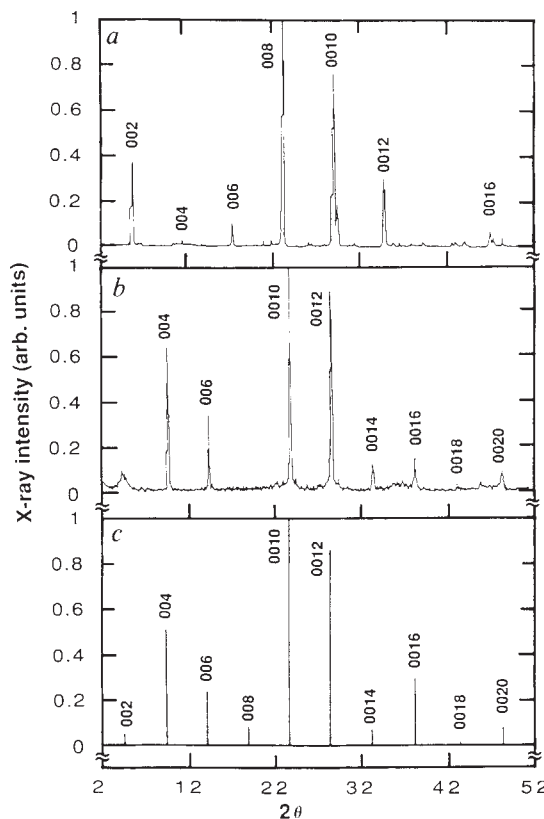


FIG. 1 Highly oriented crystal X-ray diffraction patterns of *a*, pristine $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_8$ and *b*, $\text{IBi}_2\text{Sr}_2\text{CaCu}_2\text{O}_y$. *c*, Predicted diffraction pattern of $\text{IBi}_2\text{Sr}_2\text{CaCu}_2\text{O}_y$ assuming that the iodine is intercalated between [Bi-O] bilayers.

the resulting materials were mixtures of stage-1 and higher-stage phases. Single-phase stage-1 materials were obtained for temperatures in the range 150–200 °C and times of 10–15 days. Unlike most graphite intercalation compounds, the iodine-intercalated oxide superconductor is stable in air, which greatly simplifies characterization of physical properties and may be useful in applications. To determine the amount of iodine introduced into the host material, we studied both the weight change and scanning-electron-microscope X-ray fluorescence of the single-phase stage-1 material. Both methods yielded a 1:1 ratio of I to Ca, that is, the resulting material is $\text{IBi}_2\text{Sr}_2\text{CaCu}_2\text{O}_y$.

The X-ray diffraction patterns of highly oriented (along the c axis) crystals of $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_8$ and $\text{IBi}_2\text{Sr}_2\text{CaCu}_2\text{O}_y$ are shown in Fig. 1a and b, respectively. The c -axis unit-cell dimension for the pristine material is 30.82 Å, whereas that for $\text{IBi}_2\text{Sr}_2\text{CaCu}_2\text{O}_y$ is 37.78 Å, corresponding to an increase of ~ 7 Å, or 23%. The $\text{IBi}_2\text{Sr}_2\text{CaCu}_2\text{O}_y$ material is stage-1 and single-phase to a resolution better than 1%. Because samples intercalated over a period of 10 days have the same structure as samples intercalated over 15 days, the structures are most likely fully saturated.

For each unit cell of host $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_8$ there are two intercalated layers of iodine; each iodine layer expands the c axis by ~ 3.5 Å. To determine whether the intercalated layer lies between the [Bi–O] planes or between the [Cu–O] planes, we calculated the expected relative X-ray scattering intensities for both possibilities. We obtained good agreement between the predicted and experimental intensities by assuming that the iodine layer is between the weakly bound [Bi–O] bilayers. Figure 1c shows the calculated intensity for this configuration; the results should be compared to the experimental data in Fig. 1b. The interlayer [Bi–O]–[Bi–O] spacing is the largest in the $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_8$ structure (10% larger than the Cu–O interlayer spacing) and is thus the most receptive site for iodine intercalation.

Although iodine intercalation dramatically increases the c -axis unit-cell dimension in $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_8$, it has little effect on the in-plane a and b dimensions. Figure 2 shows an X-ray powder diffraction pattern for iodine-intercalated polycrystalline $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_8$. The c -axis unit-cell dimension is 37.8 Å (in agreement with the intercalated single-crystal data), whereas the a and b dimensions are both 5.4 Å, equal to the a and b dimensions of the pristine material³.

We have characterized the superconducting properties of $\text{IBi}_2\text{Sr}_2\text{CaCu}_2\text{O}_y$ by magnetization measurements and, to a lesser extent, by transport measurements. Figure 3 shows the temperature-dependent a.c. magnetic susceptibility χ_{ac} for a single crystal of $\text{IBi}_2\text{Sr}_2\text{CaCu}_2\text{O}_y$, obtained at 10 MHz. A sharp superconducting transition is observed at 80 K; this value was consistently obtained for different specimens of $\text{IBi}_2\text{Sr}_2\text{CaCu}_2\text{O}_y$, independent of the original T_c of the host material (which ranged

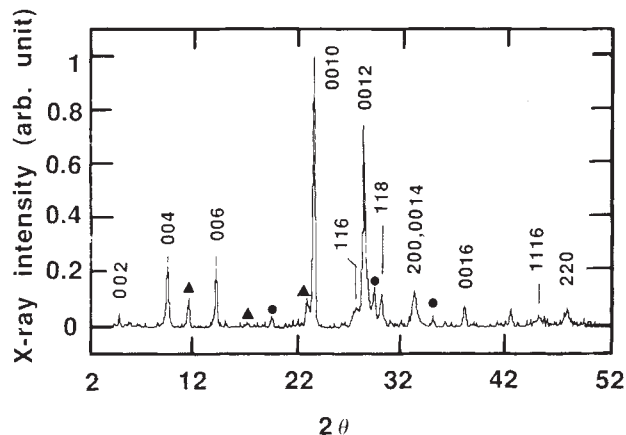


FIG. 2 Powder X-ray diffraction pattern for $\text{IBi}_2\text{Sr}_2\text{CaCu}_2\text{O}_y$ indexed to the double-slab unit cell. The triangles and circles identify very small amounts of impurity phase.

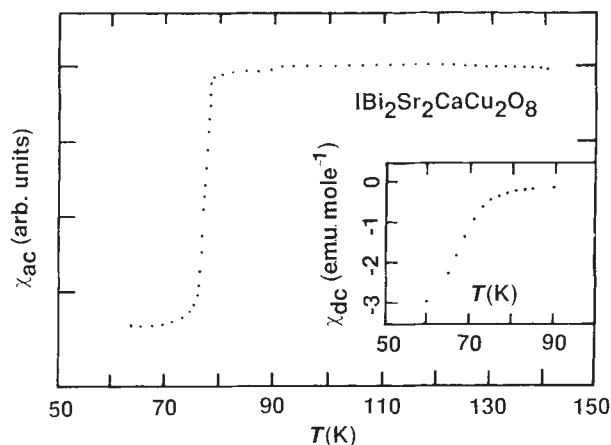


FIG. 3 A.c. magnetic susceptibility χ_{ac} of $\text{IBi}_2\text{Sr}_2\text{CaCu}_2\text{O}_y$. The transition temperature is 80 K. The inset shows the d.c. magnetic susceptibility χ_{dc} near the transition region; the data indicate bulk superconductivity.

from 82 to 90 K). To estimate the superconducting volume fraction in the intercalated material, χ_{dc} was measured using a d.c. SQUID (superconducting quantum interference device). The inset in Fig. 3 shows χ_{dc} data near the superconducting transition. At low temperatures, $\sim 30\%$ of an ideal d.c. Meissner effect is obtained, comparable to that observed for the pristine material. This indicates that $\text{IBi}_2\text{Sr}_2\text{CaCu}_2\text{O}_y$ is a bulk superconductor. Preliminary measurements of the electrical resistivity, performed along the ab plane of $\text{IBi}_2\text{Sr}_2\text{CaCu}_2\text{O}_y$, showed a nearly linear temperature-dependence in the normal state above T_c (characteristic of the ab -plane behaviour of host compound). The resistivity dropped to zero at T_c .

The band structure⁴ of $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_8$ shows that the density of states at the Fermi energy $N(E_f)$ is dominated by bands derived from the Cu(3d)–O(2p) orbitals. Thus, as we expect that the iodine only perturbs the Bi–O bands, there should be a very small effect on $N(E_f)$ and, in turn, on T_c . We would expect a much larger ΔT_c if iodine were intercalated between the [Cu–O] planes. Therefore, band-structure calculations are consistent with our determination of the iodine placement between the [Bi–O] layers.

Based on many studies of the copper oxide superconductors, it has been suggested (see, for example, ref. 5) that only the copper-oxygen planes (square, pyramidal and octahedral) are critical structural components in these high- T_c systems. The secondary structural components are expected to act only as 'charge reservoirs' which control the local charge concentration on the copper-oxygen planes. The superconducting transition temperature is closely related to the effective valence of the Cu atoms in the plane⁵.

Assuming that the Bi–O planes do serve as charge reservoirs for the [Cu–O] planes, we would expect iodine intercalation to increase the concentration of holes and therefore increase the carrier concentration and T_c . The observed change in T_c , however, is small and negative, suggesting that the intercalated iodine is not electrically active. It is possible that molecular iodine was intercalated, but further measurements are needed to confirm this.

Knowing the placement of the intercalated iodine layer and the resulting T_c allows us to compare our results with the predictions of theoretical models that focus on interlayer coupling in the high- T_c oxides. The model of Wheatley *et al.*⁶ is based on a combination of near-[Cu–O]-plane coupling and next-cell coupling, whereas Ihm and Yu⁷ have proposed a model in which only near [Cu–O] planes interact. According to these models, intercalation between the consecutive [Cu–O] layers in $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_8$ (doubling the spacing there) would reduce T_c drastically, but intercalation between the [Bi–O] layers would change T_c by less than 5 K. Intercalation of the thallium-based

oxide superconductors would distinguish between these models, because the model of Ihm and Yu predicts no change in T_c for intercalation between the [TI-O] layers, whereas the model of Wheatley *et al.* predicts that T_c would fall sharply.

Another interesting question addressed by intercalation of high- T_c superconductors is whether or not the superconductivity in these materials is two-dimensional, as has been suggested by other experimental studies^{8,9}. An excellent example of two-dimensional superconductivity is the TaS₂ intercalation compound with expansion of the *c*-axis by a few angstroms up to 60 Å (by insertion of organic compounds into host materials)¹. These compounds still remain superconducting which suggests that the superconductivity is predominantly two-dimensional. In the case of IBi₂Sr₂CaCu₂O_y, although there is still insufficient data to draw a firm conclusion, the fact that the insertion of 3.5-Å iodine layer does not drastically affect the T_c of the pristine material implies two-dimensional character of the superconducting processes in this material. □

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1. Levy, F. (ed.) *Intercalated Layered Materials* (Reidel, Dordrecht, 1979).
2. Lindberg, P. A. P. *et al. Phys. Rev. B* **39**, 2890–2893 (1989).
3. Yoon, K. & Francois, M. Z. *Phys. B* **76**, 413–444 (1989).
4. Herman, F., Kasowski, R. V. & Hsu, W. Y. *Phys. Rev. B* **38**, 204–207 (1988).
5. Cava, R. J. *Science* **247**, 656–662 (1990).
6. Wheatley, J. M., Hsu, T. C. & Anderson, P. W. *Nature* **333**, 121 (1988).
7. Ihm, J. & Yu, D. B. *Phys. Rev. B* **39**, 4760–4763 (1989).
8. Balestrino, G., Nigro, A., Vaglio, R. & Marinelli, M. *Phys. Rev. B* **39**, 12264–12266 (1989).
9. Lowndes, D. H., Norton, D. P. & Budai, J. D. *Phys. Rev. Lett.* **65**, 1160–1163 (1990).

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Peroxy radicals from night-time reaction of NO₃ with organic compounds

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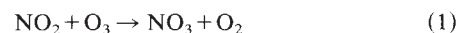
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REACTIONS of free radicals are the driving force for most chemical processes in the atmosphere. Although hydroxyl (OH) and nitrate (NO₃) radicals are the most reactive, peroxy radicals such as HO₂ and CH₃O₂ have an important role as intermediates in tropospheric ozone generation and as precursors to OH radicals¹. Moreover, self-reaction of peroxy radicals is the only known gas-phase source of peroxides to the atmosphere. It has long been known that there exist pathways to the formation of radicals—in particular NO₃ (refs 2–5)—that do not require sun-

light, yet radical chemistry is usually discussed in terms of photochemistry occurring during the day¹. The possibility of night-time production of peroxy radicals has received little attention, except perhaps in highly polluted air^{6–8}. Here we show that recent kinetic investigations^{9,10} combined with field observations of NO₃, organic and other relevant species^{11,12} suggest that tropospheric high night-time concentrations of peroxy radicals—possibly even exceeding daytime values—may occur under certain atmospheric conditions, for instance in coastal areas or forests and in air masses affected by urban emission or biomass burning. The prerequisite for this occurrence is the simultaneous presence in the same air mass of reactive organic species, oxides of nitrogen and ozone.

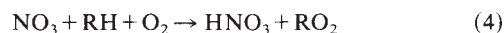
The chemical processes we discuss here are initiated by reaction of NO₂ with ozone to form NO₃ radicals



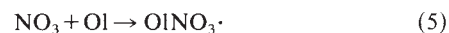
Depending on the trace-gas composition of the air mass, several reactions can generate peroxy radicals (see Table 1). For instance, they are generated by the reactions of NO₃ with aldehydes



In both cases, the rate-limiting step is the abstraction of the hydrogen atom by NO₃ from the aldehyde molecule. Similarly, H-atom abstraction from alkanes (RH) by NO₃ leads to RO₂ radical formation



Typically, however, these reactions are relatively slow (see Table 1). Although NO₃ radicals react considerably faster with olefins (OI), the products of those reactions are not very well characterized at present. The most likely reaction sequence is initiated by NO₃ addition¹³



The resulting adduct is assumed to add O₂, forming a peroxy radical.

In air masses containing dimethyl sulphide, such as coastal or maritime sites, the most important loss process of NO₃ can be reaction with dimethyl sulphide (DMS)¹⁴. Reactions (6a, b) would produce a significant number of RO₂ radicals



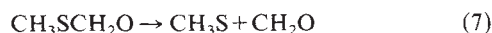
Jensen *et al.*¹⁵ tentatively concluded that reaction (6b) should dominate. On the other hand, there are several indications that reaction (6a) should not be excluded *a priori*, in particular because the S—C bond is probably weaker than the S—ONO₂ bond. Other experiments¹⁶ have shown O-atom transfer to be unlikely. In either case, the radicals produced in reactions (6a) or (6b) will immediately add O₂ to yield the peroxy radicals CH₃O₂ or CH₃SCH₂O₂, respectively. Reaction of the peroxy

TABLE 1 NO₃ reactions leading to RO₂ formation

Reacting species X	Typical mixing ratio (p.p.t.)	$k_{X+\text{NO}_3}$ (cm ³ molec ⁻¹ s ⁻¹)	RO ₂ production rate (molec cm ⁻³ s ⁻¹)	Reference
CH ₂ O	200–2,000	6.0×10^{-16}	1.5×10^3 – 1.5×10^5	18
CH ₃ CHO	10–1,000	2.7×10^{-15}	3.3×10^2 – 3.3×10^5	18
<i>n</i> -C ₄ H ₁₀	30–1,000	6.5×10^{-17}	24–800	25
C ₂ H ₄	50–400	2.1×10^{-16}	126–1,000	
C ₃ H ₆	10–200	9.4×10^{-15}	1.2×10^3 – 2.3×10^4	18
Isoprene	20–600	6.5×10^{-13}	1.6×10^5 – 4.8×10^6	13
1-C ₄ H ₈	10–200	1.2×10^{-14}	1.5×10^3 – 3.0×10^4	25
α-Pinene	8–240	6.2×10^{-12}	6.2×10^5 – 1.8×10^7	13
CH ₃ SCH ₃	1–2,000	1.0×10^{-12}	1.2×10^4 – 2.5×10^7	18

* For NO₃ concentration of 5×10^8 molec cm⁻³.

radicals with NO_3 [see reaction (13)] leads to CH_3O or $\text{CH}_3\text{SCH}_2\text{O}$ radicals, the latter will decompose



The CH_3S intermediate is believed to undergo two-step oxidation by NO_2 or O_3 , and, less likely¹⁷, by O_2 , to yield (via CH_3SO) CH_3SO_2 radicals, which may decompose



Ultimately, just as in reaction (6a), the reaction sequence initiated by reaction (6b) would produce CH_3 radicals, and hence CH_3O_2 .

Peroxy radicals (HO_2 , CH_3O_2 , $\text{CH}_3\text{SCH}_2\text{O}_2$...) formed in the reaction sequences discussed above will undergo one of the following reactions

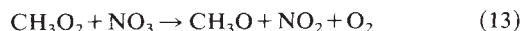


→ other products

In the case of HO_2 radicals



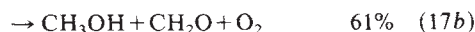
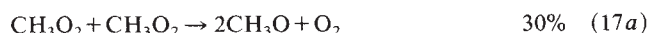
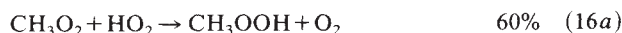
The results of Mellouki *et al.*⁹ and Crowley *et al.*¹⁰ indicate that methyl peroxy radicals and other peroxy radicals are quite likely to react in an analogous way



As reaction (9) can only occur during the day or under ozone-free night-time conditions, it will be negligible in the presence of NO_3 . Because the rate constants of reactions (9) and (10) are roughly the same (see Table 2), we can draw the interesting conclusion that the well-known conversion of RO_2 to RO by NO would be complemented by reaction (10) at night. In the RO_2 - RO conversion, and thus in HO_2 production [see reaction (19)], night-time NO_3 has the same role as NO during the daylight hours. Both reactions (9 and 10) require NO_x (reactive nitrogen compounds), whereas under conditions of low atmospheric NO_x , recombination reactions of RO_2 radicals become important



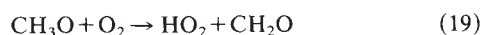
In the particular cases of HO_2 or CH_3O_2



The reaction of RO_2 with NO_2



is probably of little importance because RO_2NO_2 species are unstable under tropospheric conditions; room-temperature lifetimes of those species are typically of the order of seconds¹⁸. The RO radicals produced in reactions (9), (10) or (17a) will react with O_2 or NO_2 , or decompose. For instance



In summary, the rate-limiting step in the reaction mechanisms producing RO_2 and RO radicals is the reaction of NO_3 with organic species (see Table 1). Rapid consecutive reactions lead to RO_2 radicals, which are either lost by self-reaction, or reaction with NO_3 . In subsequent steps, HO_2 radicals are produced, being converted in part to OH by reaction (11).

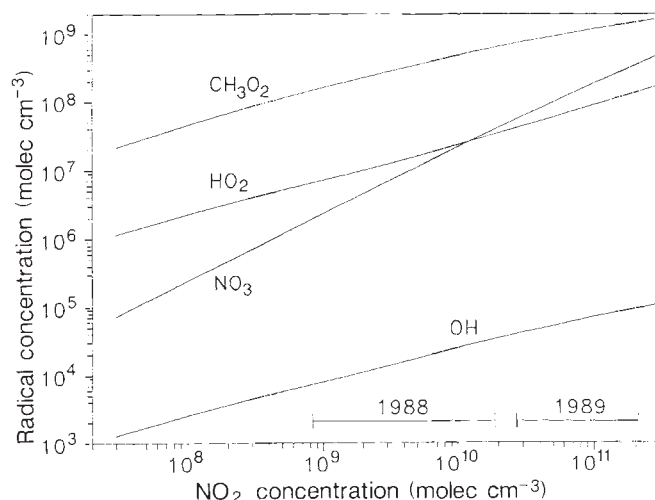


FIG. 1 Model-calculated night-time concentrations of CH_3O_2 , HO_2 , OH and NO_3 radicals as a function of NO_2 . Concentration ranges of NO_2 encountered during two measurement campaigns in Brittany (France) in 1988 and 1989 (ref. 11, and T. Brauers, personal communication), respectively, are marked by horizontal bars. Temperature, ozone and dimethylsulphide mixing ratios were held fixed at 295 K, 40 p.p.b., and 0.5 p.p.b., respectively. The resulting HO_2 levels are comparable to calculated day-time values, whereas CH_3O_2 (RO_2) radical concentrations even exceed daytime levels predicted by photochemical models. At high NO_2 levels, OH production rates are only one order of magnitude below mean daytime values, but OH concentrations remain relatively low.

In the following, we shall use the NO_3 -dimethylsulphide reaction scheme, as outlined above, as an example of the general mechanism we propose. We note, however, that the production of peroxy radicals is likely to be similar for other organic species, if their reactivity (the product of concentration and rate constant with NO_3) is the same. If we assume CH_3 radicals to be a product of reaction (6), and reaction (6) to be the rate-limiting step, then in air masses such as those observed during the OCEANO-NOX campaigns 1988 and 1989 off the coast of Brittany (typically 2 parts per 10^9 (p.p.b.) CH_2O , 0.5–0.2 p.p.b. dimethylsulphide¹² and up to 20 parts per 10^{12} (p.p.t.) NO_3 (ref. 11 and T. Brauers, personal communication)), peroxy radicals can be produced at rates $>10^7 \text{ cm}^{-3} \text{ s}^{-1}$. This is almost three orders of magnitude higher than the rate of HO_2 formation ($1.5 \times 10^4 \text{ cm}^{-3} \text{ s}^{-1}$, Table 1) from reaction (2).

A time-dependent box model (see Table 2) was used to simulate the night-time chemistry of the CH_3SCH_3 - NO_x - RO_2 system. Figure 1 gives the model assumptions and results. The resulting HO_2 levels are comparable to calculated daytime values¹. Exceptionally high concentrations of CH_3O_2 radicals are calculated, exceeding daytime RO_2 levels expected from classic photochemical model calculations. Although the values for the rate of OH production from reaction (11) range up to $1.6 \times 10^5 \text{ cm}^{-3} \text{ s}^{-1}$, only one order of magnitude below mean daytime values, OH concentrations remain relatively low ($\sim 10^5 \text{ cm}^{-3}$ at the highest NO_x levels). Although this is roughly two orders of magnitude below observed summer noon peak values¹⁹, NO_3 -generated OH can occur, independent of season, throughout the night. Thus the concentrations must be compared to the considerably lower daytime average OH concentrations. These night-time OH levels may well be significant for the removal of other trace gases, particularly (in mid-latitudes) in the winter.

Although there are similarities between daytime and night-time NO_x -catalysed conversion of RO_2 to RO (reactions (9) or (10)), this process will usually be less effective at night because night-time NO_3/NO_x ratios (0.01–0.1) are lower than daytime NO/NO_x ratios (~ 0.3). This is the main reason for the low calculated OH concentrations as well as the low OH/RO_2 ratios (10^{-3} , compared to $\sim 10^{-2}$ during the day). Examining the

TABLE 2 Reaction scheme for night-time NO₃-RO₂ chemistry

Reaction	Rate constant (cm ³ molec ⁻¹ s ⁻¹) (295 K, 1 atm)*
OH + CO	→ H + CO ₂ 2.4 × 10 ⁻¹³
OH + CH ₄	→ H ₂ O + CH ₃ 8.3 × 10 ⁻¹⁵
OH + H ₂	→ H ₂ O + H 6.3 × 10 ⁻¹⁵
OH + O ₃	→ HO ₂ + O ₂ 6.6 × 10 ⁻¹⁴
OH + CH ₂ O	→ H ₂ O + CHO 1.0 × 10 ⁻¹¹
OH + NO ₂	→ HNO ₃ 1.2 × 10 ⁻¹¹
OH + CH ₃ OOH	→ H ₂ O + CH ₃ O ₂ 6.6 × 10 ⁻¹²
OH + CH ₃ OOH	→ H ₂ O + CH ₂ + O + OH 4.4 × 10 ⁻¹²
H + O ₂	→ HO ₂ 1.4 × 10 ⁻¹²
CH ₃ + O ₂	→ CH ₃ O ₂ 2.2 × 10 ⁻¹²
O ₃ + HO ₂	→ 2O ₂ + OH 2.0 × 10 ⁻¹⁵
OH + HO ₂	→ H ₂ O + O ₂ 1.1 × 10 ⁻¹⁰
CH ₃ O ₂ + NO ₃	→ CH ₃ O + NO ₂ + O ₂ 2.3 × 10 ⁻¹² ; ref. 10
CH ₃ O ₂ + CH ₃ O ₂	→ 2CH ₃ O + O ₂ 1.1 × 10 ⁻¹³ ; ref. 27
CH ₃ O ₂ + CH ₃ O ₂	→ CH ₂ O + CH ₃ OH + O ₂ 2.2 × 10 ⁻¹³ ; ref. 27
CH ₃ O ₂ + CH ₃ O ₂	→ CH ₃ OOCH ₃ + O ₂ 3.0 × 10 ⁻¹⁴ ; ref. 27
CH ₃ O ₂ + HO ₂	→ CH ₃ OOH + O ₂ 3.6 × 10 ⁻¹² ; refs 26, 27
CH ₃ O ₂ + HO ₂	→ CH ₂ O + H ₂ O + O ₂ 2.4 × 10 ⁻¹² ; refs 26, 27
CH ₃ O + O ₂	→ CH ₂ O + HO ₂ 1.9 × 10 ⁻¹⁵
CHO + O ₂	→ CO + HO ₂ 5.6 × 10 ⁻¹²
HO ₂ + HO ₂	→ H ₂ O ₂ + O ₂ 5.4 × 10 ⁻¹² †
HO ₂ + NO ₃	→ HNO ₃ + O ₂ 0.9 × 10 ⁻¹² ; ref. 9
HO ₂ + NO ₃	→ OH + NO ₂ + O ₂ 3.6 × 10 ⁻¹² ; ref. 9
NO ₃ + NO ₂	→ NO + NO ₂ + O ₂ 5.6 × 10 ⁻¹⁶ ; ref. 28
NO ₃ + NO ₂	→ N ₂ O ₅ 1.7 × 10 ⁻¹²
N ₂ O ₅	→ NO ₃ + NO ₂ 4.1 × 10 ⁻² s ⁻¹
NO ₃ + CH ₃ SCH ₃	→ products (see text) 1.0 × 10 ⁻¹²

* Data from Atkinson *et al.*¹⁸, unless otherwise noted.† [H₂O] = 3.5 × 10¹⁷ molec cm⁻³.

chemical system in detail reveals that NO₃ radicals are mostly lost by the initial reaction (with DMS, in our example), rather than by reaction with HO₂ or other RO₂. Peroxy radical loss, on the other hand, is mostly due to self-reaction, and therefore considerable production of peroxides and formaldehyde can be expected. Photolysis of these species will lead to higher radical and ozone production during the day.

It is interesting to consider the special conditions under which such high production rates of HO_x radicals are most likely to occur. From the mechanism outlined above, it is clear that elevated levels of NO_x and reactive organic species are required simultaneously with ozone. On the continents NO_x will easily be sufficiently high, but dimethylsulphide is usually low. Conversely, in maritime environments dimethylsulphide and hydrocarbons can be abundant²⁰, but usually NO_x is low²¹. High natural emission of reactive hydrocarbons can be found in forests²². Thus, the areas where high night-time radical concentrations are likely to occur are polluted coastlines, forests subject to anthropogenic pollution and urban air masses (if conditions permit the survival of ozone during the night). In fact, one of the few attempts to measure night-time RO₂ radical concentrations found up to 1.2 × 10¹⁰ cm⁻³ at a rural site in Germany²³. The above conditions favouring night-time RO₂ production are all linked to anthropogenic NO_x emission, the only natural cause of simultaneous presence of reactive hydrocarbons and NO_x seems to be biomass burning²⁴. Thus it is quite possible that the RO₂ concentration in the above-mentioned areas has increased dramatically since the beginning of industrialization. Further research may identify more areas where high night-time RO₂ concentrations can occur and may change our understanding of night-time atmospheric chemistry. □

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- Logan, J. A., Prather, M. J., Wofsy, S. C. & McElroy, M. B. *J. geophys. Res.* **86**, 7210–7254 (1981).
- Noxon, J. F., Norton, R. B. & Marovich, E. *Geophys. Res. Lett.* **7**, 125–128 (1980).
- Platt, U., Perner, D., Harris, G. W., Winer, A. M. & Pitts, J. N. *Geophys. Res. Lett.* **7**, 89–92 (1980).
- Platt, U., Perner, D., Schröder, J., Kessler, C. & Toennissen, A. *J. geophys. Res.* **86**, 11965–11970 (1981).
- Platt, U., Winer, A. M., Biermann, H. W., Atkinson, R. & Pitts, J. N. *Environ. Sci. Technol.* **18**, 365–369 (1984).

- Cantrell, C. A. *et al. J. phys. Chem.* **89**, 139–146 (1985).
- Cantrell, C. A., Davidson, J. A., Busarow, K. L. & Calvert, J. G. *J. geophys. Res.* **91**, 5347–5353 (1986).
- Carter, W. P. L. *Atmos. Environ.* **A24**, 481–518 (1990).
- Mellouki, A., LeBras, G. & Poulet, G. *J. phys. Chem.* **92**, 2229–2234 (1988).
- Crowley, J. N., Burrows, J. P., Moorgat, G. K., Poulet, G. & LeBras, G. *Int. J. chem. Kinet.* **22**, 673–681 (1990).
- Brauers, T., Dorn, H. P. & Platt, U. *Proc. 5th Eur. Symp. Physical and Chemical Behaviour of Atmospheric Pollutants* (eds Restelli, G. & Angeletti, G.) 237–242 (Kluwer, Dordrecht, 1990).
- Pashalidis, S., Carlier, P., Mouvier, G., *Proc. 5th Eur. Symp. Physical and Chemical Behaviour of Atmospheric Pollutants* (eds Restelli, G. & Angeletti, G.) 457–462 (Kluwer, Dordrecht, 1990).
- Atkinson, R. *Atmos. Environ.* **A24**, 1–41 (1990).
- Winer, A. M., Atkinson, R. & Pitts, J. N. *Science* **224**, 156–159 (1984).
- Jensen, N. R., Hjorth, J., Lohse, C., Skov, H. & Restelli, G. *Atmos. Environ.* (submitted).
- Dlugokencky, E. T. & Howard, G. J. *J. phys. Chem.* **93**, 1091–1096 (1989).
- Tyndall, G. S. & Ravishankara, A. R. *J. phys. Chem.* **93**, 2426–2435 (1989).
- Atkinson, R. *et al. J. phys. Chem. Ref. Data* **18**, 881–1097 (1989).
- Platt, U., Rateike, M., Junkermann, W., Rudolph, J. & Ehhalt, D. H. *J. geophys. Res.* **93**, 5159–5166 (1988).
- Ehhalt, D. H., Rudolph, J., Meixner, F. & Schmidt, U. *J. Atmos. Chem.* **3**, 29–52 (1985).
- Drummond, J. W., Ehhalt, D. H. & Volz, A. *J. geophys. Res.* **93**, 15831–15849 (1988).
- Roberts, J. M. *et al. Environ. Sci. Technol.* **19**, 364–369 (1985).
- Mihelcic, D., Muegen, P. & Ehhalt, D. H. *J. Atmos. Chem.* **3**, 341–361 (1985).
- Greenberg, J. P., Zimmerman, P. R., Heidt, L. & Pollock, W. *J. geophys. Res.* **89**, 1350–1354 (1984).
- Atkinson, R., Aschmann, S. M. & Pitts, J. N. *J. phys. Chem.* **92**, 3454–3457 (1988).
- Lightfoot, P. D., Veyret, B. & Lesclaux, R. *J. phys. Chem.* **94**, 708–714 (1990).
- Simon, F. G., Schneider, W. & Moortgat, G. K. *Int. J. chem. Kinet.* **22**, 791–813 (1990).
- Graham, R. A. & Johnston, H. S. *J. phys. Chem.* **82**, 254–268 (1978).

Ridge jumps and propagations in the South Atlantic Ocean

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THE normal pattern of mid-ocean-ridge divergent plate boundaries comprises straight spreading segments with narrow zones of crustal accretion, bounded by transform faults where these segments are offset. Normally the configuration of spreading segments and transform faults is stable for long periods, but in places on fast and medium-rate spreading ridges the transform offsets have propagated along the ridge axis as some spreading segments have grown at the expense of adjacent ones^{1–7}. Although such behaviour has hitherto not been well documented on slow-spreading ridges, we have mapped multiple ridge jumps and propagating rifts in two areas of the slow-spreading (18 mm yr⁻¹ half rate) South Atlantic ridge crest. We attribute this anomalous behaviour in the South Atlantic to the presence of hotspots near our study areas, causing elevated asthenospheric mantle temperatures and lateral mantle flow, with consequent excess volcanism and disruption of the normally well focused melt delivery processes at the ridge crest.

Two areas of the Mid-Atlantic Ridge (MAR) spreading centre were surveyed from the RV *Conrad* using Sea Beam bathymetry, and magnetic and gravity measurements. The detailed surveys are of areas for which an aeromagnetic survey has provided the regional tectonic framework⁸. Both areas lie above the extended mushroom heads of anomalously hot mantle surrounding mantle plumes⁹ although the central plumes themselves lie to the east of the ridge crest (Fig. 1). The St Helena plume is 400–600 km east of the survey at 16° S whereas the Ascension plume is much closer to the ridge crest, lying only 100–200 km east of the survey at 9° S⁸.

The sea-floor spreading anomalies shown in Figs 2 and 3 were identified from magnetic profiles and a three-dimensional inversion to magnetization (Figs 2b, 3b) which removes skewness and topographic effects^{10,11}. Anomalies from the neovolcanic zone (labelled 0) exhibit magnetizations three to five times larger than the older magnetic anomalies from more-altered rock. The propagating rifts leave prominent V-shaped scars in the bathymetry known as pseudo-faults¹², which correlate with offsets in the magnetic anomalies and breaks in the magnetization pattern. Where ridge jumps have occurred the resultant magnetic anomaly pattern can be complex, but identification of the ridge jumps is aided by the Sea Beam data which shows inward-facing

slopes interpreted as normal faults on either side of the extinct ridges.

The locus of rifting and volcanism in the survey near the Ascension hotspot has been highly variable over the past 5 Myr, producing several eastward ridge jumps of up to a few tens of kilometres each and a prominent northward-propagating rift (Fig. 2). Our interpretation of the present configuration is shown in Fig. 2 and the evolutionary sequence that generated it is illustrated in Fig. 4. Before anomaly-3' time (~ 5.5 Myr ago) the ridge formed an unbroken linear segment⁸. The spreading axis south of 9°S subsequently migrated eastward to create a small left-lateral offset by anomaly-3 time and then began propagating northward at a rate similar to the half-spreading rate (Fig. 4a). Between 1.5–1.0 Myr it jumped a further 15 km (Fig. 4b), increasing the offset of the propagator and abandoning the indistinct rift between anomalies 2W (west) and 2E (east). Most recently, the ridge jumped another 35 km eastward and increased its northward propagation rate dramatically (Fig. 4d). Individual neovolcanic segments overlap by typically 10 km and unlike normal segments of the MAR there is no consistent median valley. The constructional volcanic ridges are much larger than seen in other slow-spreading areas, being typically 1,000 m high, 30–40 km long and 5 km wide. They rise to a consistent minimum depth of 1,500 m, which is much shallower than elsewhere on the MAR and reflects the enhanced melt availability here. A large caldera 300–400 m deep topping a circular volcanic platform has formed at 7°55'S.

The St Helena propagating ridge mapped near 16°S (Fig. 3) displays classic V-shaped pseudo-fault troughs. It propagated steadily southwards at ~ 24 mm yr⁻¹ from 5 Myr until 0.8–0.5 Myr ago, without the multiple ridge jumps seen in the Ascension area. The topography behind the propagator is anomalously shallow, although not to the same degree as that behind the Ascension propagator, and exhibits considerably less relief than in the Ascension area. These differences point to a reduced influence of the St Helena plume which lies several hundred kilometres further from the ridge crest than does the Ascension plume (Fig. 1). The neovolcanic zone (anomaly 0 in Fig. 3) marking the locus of most recent volcanism apparently crosses the pseudo-fault at 13°17' W. It extends from the large seamount at 16°00' S which reaches to within 1,000 m of the sea surface and crosses the sheared zone at 15°50' S. We cannot tell

whether this is just a temporary dyke overshoot or whether it marks the onset of northward rather than southward propagation. The high magnetization of the neovolcanic zone persists for only a few tens of thousands to a hundred thousand years²² before the magnetization is reduced by weathering, so it represents only a snapshot of the most recent extrusive zones. Over the million years or more between the major magnetic field reversals that give rise to the numbered sea-floor spreading anomalies, temporary dyke overshoots may occur in both directions. But there may be no permanent record of them, because their duration is short compared to the long-term average recorded by the sea-floor spreading anomalies.

We suggest that the occurrence of ridge jumps and propagators on a slow-spreading ridge is due to unusually hot underlying mantle fed laterally from adjacent mantle plumes. On normal segments of the ridge crest, away from the hotspots, the zone of intrusion is narrow (1–5 km). Melt formed by adiabatic decompression of mantle beneath the rift zone is focused into this narrow linear region. Although the melt-focusing mechanism beneath the ridge crest is still a matter for debate^{13–16}, it is clear that it derives ultimately from the interplay between partial melting and mantle flow caused by plate separation. Near an off-axis hotspot the lateral flow of mantle in the mushroom head surrounding the plume disrupts the normal stable pattern of symmetric mantle flow beneath the spreading ridge axis, and therefore disrupts the normal focusing mechanism. This, coupled with the enhanced melt availability and the thinner and weaker lithosphere caused by elevated mantle temperatures, fosters off-axis extrusion and jumps in the neovolcanic zone.

The ridge crest in the St Helena survey, although slow-spreading, behaves more like a medium- or fast-spreading ridge. It exhibits a continuously propagating rift and relatively smooth topography, with a small median valley. Although the St Helena plume lies 400–600 km away, the flow of solid but ductile mantle away from the central plume produces a large region of anomalously hot and geochemically distinct¹⁷ mantle. In the distal regions of the mushroom head such as the ridge crest at 16°S the mantle is probably only a few tens of degrees Celsius hotter than normal⁹. But even an increase of as little as 30°C above normal is sufficient to increase the volume of melt produced by decompression under an oceanic rift by about 30%^{18,19}. The rate of supply of melt to normal spreading centres is proportional to the spreading rate, with the intervals between pulses of magma injection being much shorter on fast than on slow-spreading ridges. The above-normal rate of melt supply on slow-spreading ridges near hotspots may make them behave more like faster-spreading ridges.

The survey at 9°S displays a more extreme example of the effect of a hotspot, because the ridge crest is within 100–200 km of the Ascension mantle plume. The mantle is therefore hotter than beneath the 16°S survey and a greater excess of melt is produced by decompression beneath the spreading centre producing rugged topography in the form of large constructional volcanic ridges. Ridge jumps and off-axis extrusion are common, reflecting the influence of horizontal flow in the mushroom head of the hotspot with rates similar to the normal mantle flow rates caused by plate separation. In addition, extra melt is probably produced off-axis as the mantle from the Ascension plume spreads laterally beneath the overlying lithospheric plate. As it migrates upwards along the base of the rapidly thinning lithosphere approaching the spreading centre, it decompresses and partially melts. This produces large off-axis seamounts to the east of the ridge axis, one of which (at 9°45' S) rises to within 72 m of the sea surface. Again, there are prominent geochemical anomalies along the ridge crest¹⁷, indicating that the melt has formed from mantle that was fed originally by a hotspot.

The ridge jumps are consistently to the east, towards the Ascension mantle plume and therefore into weaker lithosphere. The timing of ridge jumps may be caused by episodic nature of the flow of the mantle plumes. The propagating rifts in both

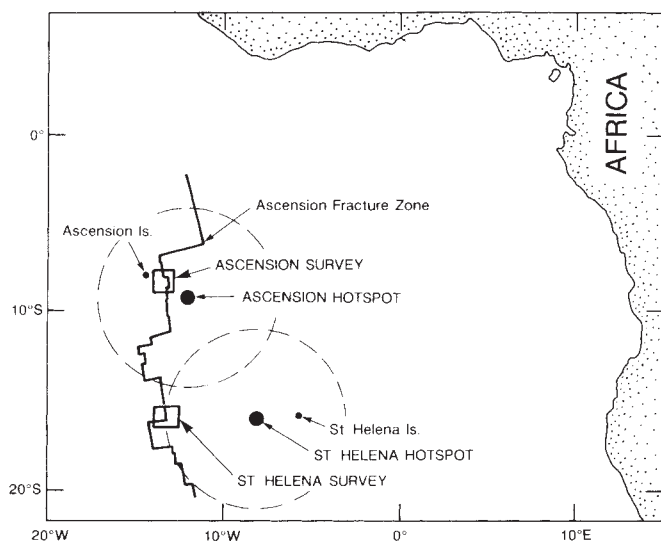


FIG. 1 Location map of the two study areas (rectangles) from which extracts are shown in Figs 2 and 3. Dots show presumed present locations of the Ascension and St Helena mantle plumes. Circles of 500-km radius indicate the probable extent of anomalously hot asthenospheric mantle emplaced from the narrow (~ 50 -km radius) mantle plumes.

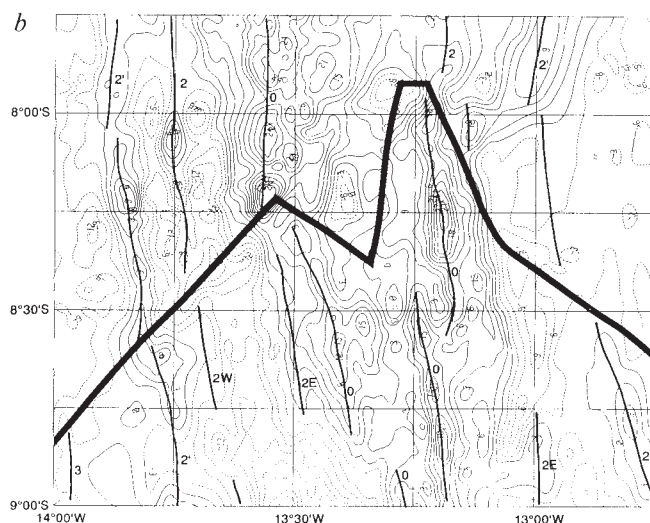
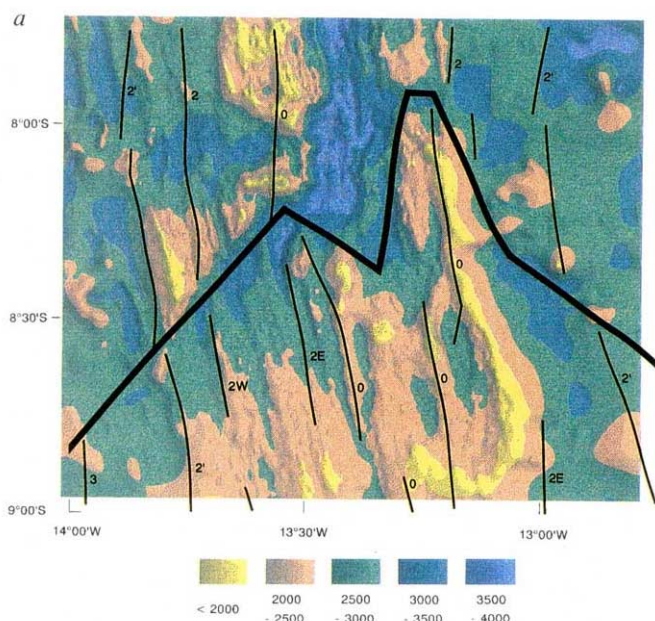


FIG. 2 *a*, Shaded relief map of Ascension propagator using bathymetry from a Sea Beam survey recorded by the Naval Research Laboratory combined with historical shipboard data. Illumination is from the east with a colour contour interval of 500 m. Thin black lines show magnetic-anomaly isochrons. Heavy lines denote traces of pseudo-faults generated by the propagating rift. As it propagated northward, the southern (growing) ridge segment jumped eastward twice, increasing the offset between the growing and doomed rift from the original 6 km to ~15 km and most recently to 50 km (see Fig. 4). An extinct rift lies between anomaly 2W (west) and 2E (east) isochrons. Rapid northward propagation of the new axis during the past million years has disrupted the outer pseudo-fault. There is a large caldera

near the spreading axis at 7°55' S. *b*, Contour map of magnetization obtained by three-dimensional inversion of the magnetic and bathymetric data^{10,11} with numbered magnetic-anomaly lineations marked on the centres of the normally magnetized anomalies. Lineations labelled 0 show highly magnetized axes, which are diagnostic of the recently erupted, relatively unweathered basalts of neovolcanic zones. Very high magnetization is diagnostic of recently erupted, relatively unweathered basalts. Spreading on the axis marked 0 just to the east of 2E is probably dying out and being supplanted by the easternmost system of en echelon lineations marked 0, which exhibit markedly higher magnetizations. Contour interval is 3 A m⁻¹ with solid and broken lines for positive and negative anomalies, respectively.

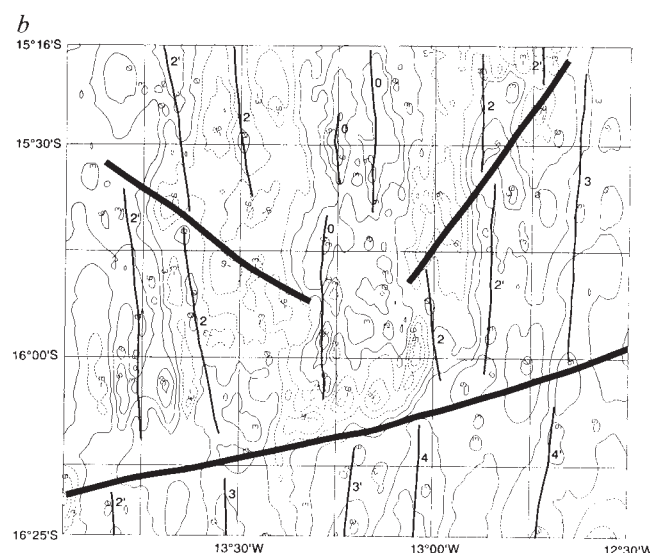
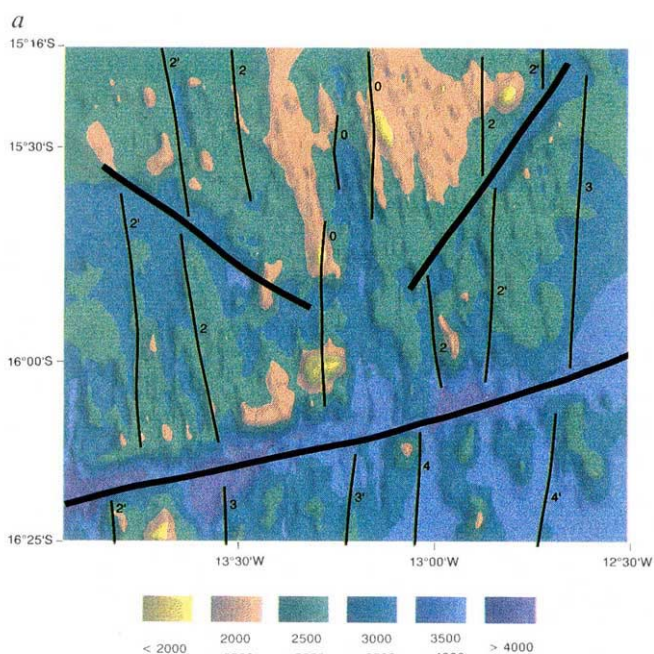


FIG. 3 *a*, Shaded relief map of the St Helena propagator constructed in the same manner as Fig. 2*a*. The approximately east-west heavy black line towards the bottom of the map shows a fracture zone. The most recent locus of extrusion marked by the neovolcanic zone (labelled 0) at 13°17' W

may cross the pseudo-fault, joining two prominent seamounts caused by excess volcanism. *b*, Contour map of magnetization constructed in the same way as Fig. 2*b*.

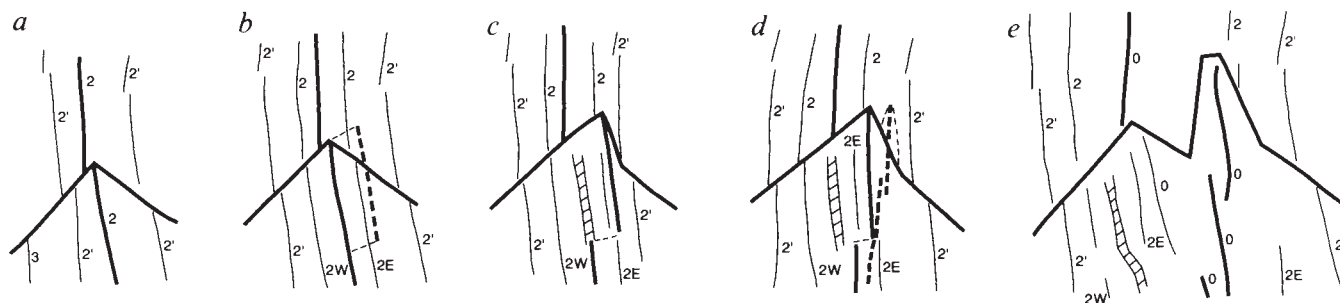


FIG. 4 Evolution of the Ascension propagator: *a*, ~1.9 Myr (anomaly 2), the southern ridge segment has been propagating northward since at least anomaly-3 time; *b*, ~1.5 Myr, a portion of the propagating southern rift segment jumps eastward leaving a piece of the eastern anomaly 2 (African crust) attached to the South American plate; *c*, ~1.2 Myr, the jumped

segment continues to propagate northward, leaving an abandoned spreading centre (hatched); *d*, ~0.8 Myr, the southern segment jumps eastward again and the rate of northern propagation increases; *e*, present ridge configuration. The evolution of the St Helena propagator has been much simpler, with no ridge jumps, and can be understood readily from Fig. 3a.

survey areas move away from the hotspot, a behaviour also reported for the vicinities of other hotspots^{1-3,5,7,20}.

A similar response of multiple ridge jumps and large propagating rifts is seen in back-arc regions such as the Lau Basin²¹. Again, this behaviour may be caused by excess melt availability and the disruptive effect of lateral mantle flow, although in the case of back-arc basins the extra melt and induced mantle flow is caused by plate subduction rather than by hotspots.

We conclude that the presence of hotspots markedly alters the accretionary processes at slow-spreading ridge crests, even though the mantle plumes may be many hundreds of kilometres away. The narrow well focused accretionary processes become progressively more disrupted as the hotspots lie closer to the spreading axis and both the mantle temperatures, with resultant enhanced melt generation, and the horizontal mantle flow rates become higher. □

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1. Hey, R. *Earth planet. Sci. Lett.* **37**, 321-325 (1977).
2. Hey, R. & Vogt, P. *Tectonophysics* **37**, 41-52 (1977).
3. Johnson, H. P., Karsten, J. L. & Delaney, J. R. *J. geophys. Res.* **88**, 2297-2315 (1983).
4. Lonsdale, P. *J. geophys. Res.* **88**, 9393-9406 (1983).
5. Naar, D. F. & Hey, R. N. *J. geophys. Res.* **91**, 3425-3438 (1986).
6. MacDonald, K. C., Haymon, R. M., Miller, S. P., Sempere, J.-C. & Fox, P. J. *J. geophys. Res.* **93**, 2875-2898 (1988).
7. Karsten, J. L. & Delaney, J. R. *J. geophys. Res.* **94**, 700-712 (1989).
8. Brozena, J. M. *J. geophys. Res.* **91**, 497-510 (1986).
9. Courtney, R. C. & White, R. S. *Geophys. J. R. astr. Soc.* **87**, 815-868 (1986).
10. MacDonald, K. C., Miller, S. P., Huestis, S. P. & Spiess, F. N. *J. geophys. Res.* **85**, 3670-3680 (1980).

11. Parker, R. L. & Huestis, S. P. *J. geophys. Res.* **79**, 1587-1593 (1974).
12. Scott, D. R. & Stevenson, D. J. *J. geophys. Res.* **94**, 2973-2988 (1989).
13. Whitehead, J. A., Dick, H. B. & Schouten, H. *Nature* **312**, 146-148 (1984).
14. Buck, W. R. & Su, W. *Geophys. Res. Lett.* **16**, 641-644 (1989).
15. Sotin, C. & Parmentier, E. M. *Geophys. Res. Lett.* **16**, 835-838 (1989).
16. Schilling, J. G., Thompson, G., Kingsley, R. & Humphris, S. *Nature* **313**, 187-191 (1985).
17. McKenzie, D. P. & Bickle, M. *J. Petrol.* **29**, 625-679 (1988).
18. White, R. S. & McKenzie, D. P. *J. geophys. Res.* **94**, 7685-7729 (1989).
19. Phipps Morgan, J. & Parmentier, E. M. *J. geophys. Res.* **90**, 8603-8612 (1985).
20. Parson, L. M. *et al. Geology* **18**, 470-473 (1990).
21. Klitgord, K. D. *Earth planet. Sci. Lett.* **29**, 201-209 (1976).

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Hundreds of small volcanoes on the median valley floor of the Mid-Atlantic Ridge at 24-30° N

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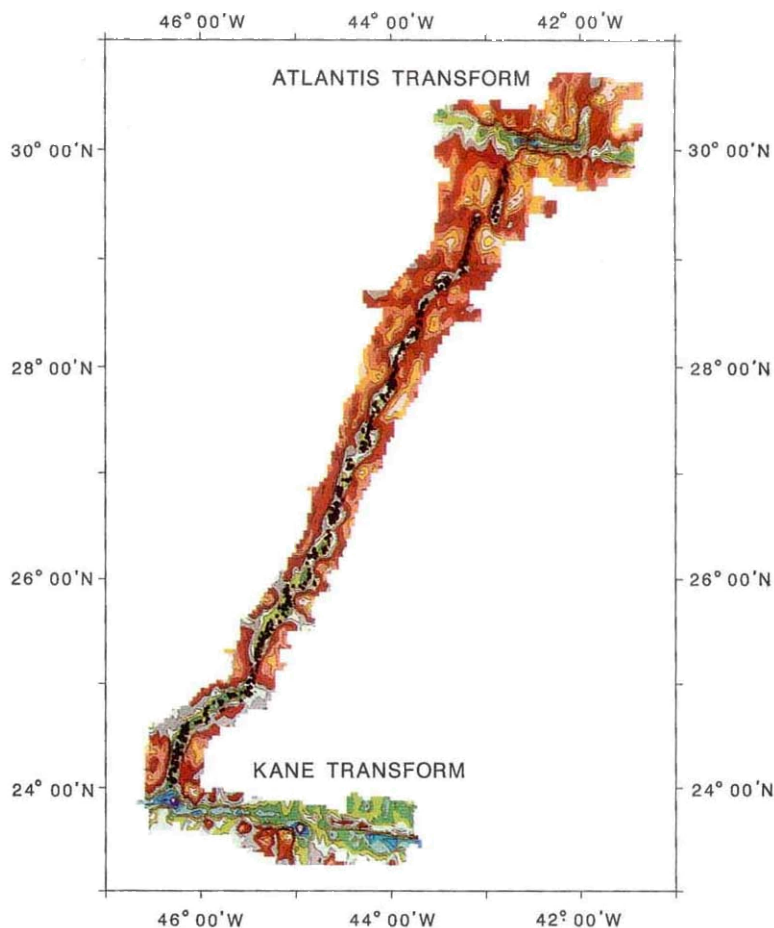
WE have identified 481 seamounts from ~6,000 km² of Sea Beam swaths of the median valley floor of the Mid-Atlantic Ridge (MAR) between 24 and 30° N. We find that seamount abundances are an order of magnitude larger (on average, 80 per 1,000 km² with relief above 50 m) than estimated in previous studies along mid-ocean ridges in the Pacific¹ and South Atlantic², suggesting that at this section of the MAR, seamount volcanism plays an important part in the formation of new oceanic lithosphere. The neovolcanic ridge, often observed on the inner valley floor³, appears to be constructed totally of individual coalesced seamounts, so that our counts represent a minimum estimate. The frequency distribution of summit heights of the MAR seamounts is consistent with the exponential model describing the heights of off-axis seamounts in the eastern Pacific⁴⁻⁶, but with different parameters. Such a distribution of heights may be a fundamental aspect of seamount volcan-

ism, related to lithospheric properties and/or magmatic plumbing. Seamount-dominated axial volcanism must create a crustal structure very different from classic fissure-fed models.

To minimize the effects of faulting on the identification of seamounts, we have restricted our investigation to the median valley floor where new oceanic crust is relatively undeformed by tectonic activity. We considered only features with their entire summit region and most of the flanks contained within the Sea Beam swaths. Using criteria specified in previous studies^{2,7}, seamounts were identified as topographic highs having a relief of greater than 50 m on all sides and having approximately equant plan shapes (aspect ratios <2). Side-scan sonar images of regions south of the Kane fracture zone indicate that most features of this type are volcanic constructions⁸. Figure 1 shows the bathymetry and locations of seamounts in the study area.

An example of the topography observed in the median valley and our geological interpretation is shown in Fig. 2. Most of the segments into which this section of the MAR is divided contain prominent volcanic ridges in the median valley³, which we take to be the sites of current crustal construction. In segments without ridges, seamounts are scattered over the median valley floor. In segments containing a volcanic ridge most seamounts are associated with the ridge and are distributed over its top and along its sides. The morphology of the volcanic ridges, which can be as high as 400 m and tens of kilometres long, is characterized by a bumpy topography on the same scale as the seamounts. We therefore infer that the ridges are constructed

FIG. 1 Map of the survey area. Sea Beam data are displayed using a 250-m contour interval. Yellow represents depths <2,500 m; red, 2,500–3,250 m; green, 3,250–4,500 m; blue, >4,500 m. Black dots are seamounts. The median valley floor is defined by the edge of the steep slopes of the median valley walls.



from piled-up seamounts (Fig. 2b), and that our counts must represent a minimum estimate of the seamount population. The shaded seamounts in Fig. 2b fully meet our counting criteria, whereas the unshaded are considered to be seamounts either overlapped by later eruptions or abutting existing terrain.

The analysis of a 800-km-long section of the median valley (which varies in width from ~5 to 11 km) yielded 481 seamounts in the height range 50–600 m (240 of which have heights ≥ 100 m), for an average density of ~80 per 1,000 km². This value is an order of magnitude larger than obtained in previous seamount studies at mid-ocean ridges. An investigation of the seamount population near the East Pacific Rise¹ between 0–24° N, on crust <10 Myr old, yielded estimates of 9 seamounts per 1,000 km², and a study along the MAR² near 26° S, on crust <7 Myr old, yielded comparable numbers (7 seamounts per 1,000 km²).

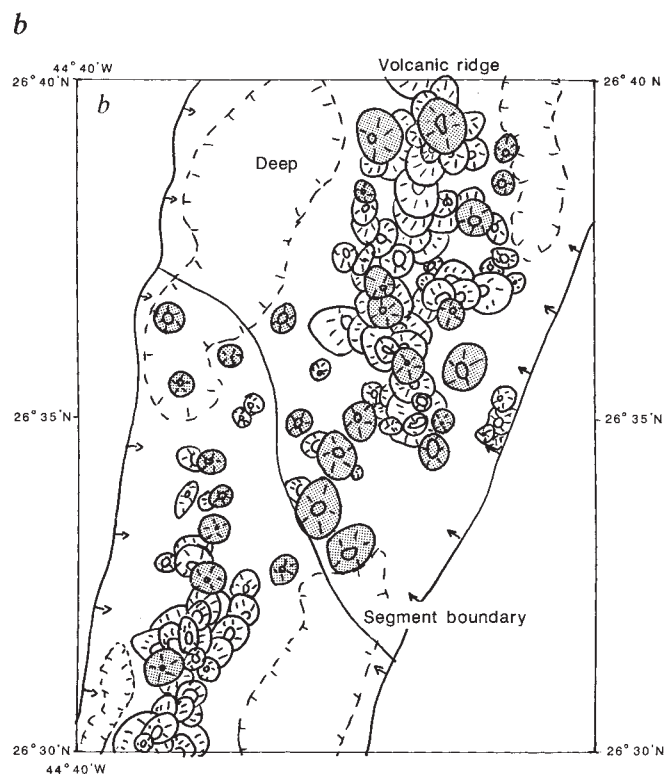
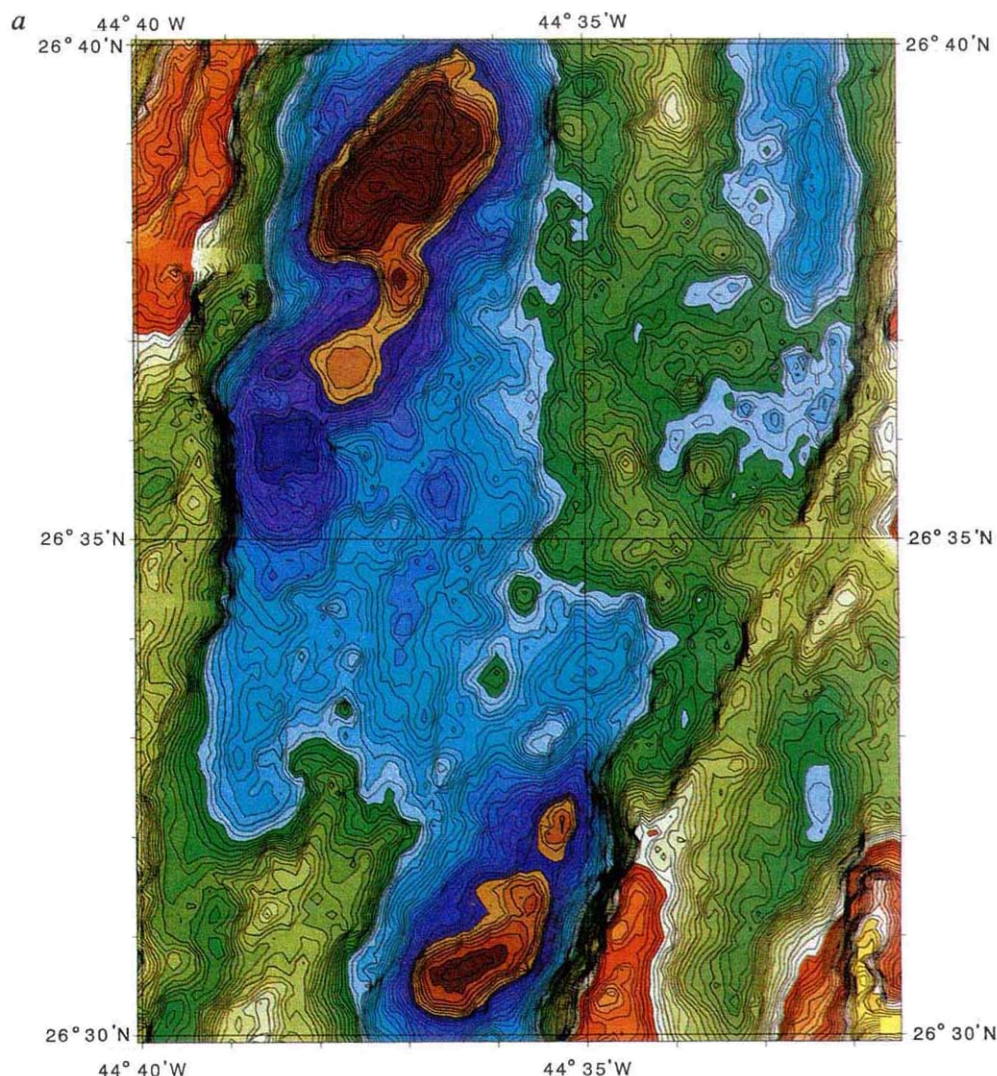
To characterize objectively the abundance and size distribution of our MAR population, we applied the analysis techniques of Jordan *et al.*⁴ and Smith and Jordan^{5,6}. This type of analysis has been undertaken for only a few areas^{4–6,9}, all of which are in the eastern Pacific. The principal conclusion of these investigations was that the seamount size distribution is very nearly exponential over a large range of summit heights^{4–6}. That is, the average number of seamounts with summit height $h \geq H$ has the value $\nu(H) = \nu_0 e^{-\beta H}$, where ν_0 is the total number of seamounts per unit area and β^{-1} is the characteristic height of the population. The summit height distribution of the seamount population on the MAR median valley floor is also consistent with the exponential model (Fig. 3). This result in itself is extremely interesting. We do not yet understand why the heights of Pacific seamounts follow an exponential distribution. (The distribution of volumes does not, for example.) That a seamount

population from a notably different tectonic environment should also exhibit an exponential height distribution indicates that there may be a fundamental control on seamount height. We suspect that the answer lies in the interaction between rising magma and the lithosphere, producing a plumbing system that controls seamount height rather than, say, volume.

Because the exponential model fits the MAR seamount counts, we can estimate population parameters ν_0 and β . From a maximum-likelihood fit of an exponential function^{5,6} to the data we obtain $\nu_0 = (19.5 \pm 0.9) \times 10^{-8} \text{ m}^{-2}$ and $\beta = (17.2 \pm 0.5) \times 10^{-3} \text{ m}^{-1}$, yielding a characteristic height of ~60 m. According to this exponential model, 1,000 km² contains an average of ~195 seamounts of all sizes. For comparison, Smith and Jordan⁶ obtained $\nu_0 = (5.4 \pm 0.6) \times 10^{-9} \text{ m}^{-2}$ and $\beta = (3.47 \pm 0.2) \times 10^{-3} \text{ m}^{-1}$ for the eastern Pacific, implying a characteristic height of ~285 m and a density of ~5 seamounts per 1,000 km². The characteristic height is several times larger for off-axis eastern Pacific crust, which is not surprising. The young, relatively thin lithosphere of the MAR median valley floor would not support large structures^{7,10,11}. The fact that the average number of seamounts is more than an order of magnitude larger for the median valley floor of the MAR is notable.

The MAR population parameters predict nearly 85×10^6 seamounts of all sizes for the whole North Atlantic, of which 25×10^5 would have heights ≥ 200 m, and 1,400 would be ≥ 500 m high. These values are far greater than others have observed in the North Atlantic^{12–15}. The discrepancy may be the result of deformation of the crust as it is transported out of the median valley. We have examined areas in the crestal mountains and find that where the intensity of faulting is high we cannot identify any seamounts. In the less-faulted areas, however, seamounts as small as 50 m high are readily recognized. There are 26

FIG. 2 *a*, Sea Beam bathymetry for a section of the MAR survey area. Contours are plotted at 20-m intervals, colour changes every 40 m. Yellow represents depths <2,900 m; red, 2,980–3,300 m; green, 3,380–3,700 m; blue, 3,780–4,100 m; brown, >4,180 m. The shapes of seamounts were approximated as flat-topped axisymmetric cones^{4–6,24}, and for each seamount, latitude, longitude, water depth to the summit, basal diameter, top diameter, height of the truncated cone and maximum summit height of the seamount were recorded. *b*, Interpretive drawing of *a*. The base of the first major scarp that begins the steps up the inner valley wall was identified on each side of the inner rift (shown as solid lines), and the cross-axis width of the median valley floor was defined to be the distance between the two. Arrows point towards the median valley floor; tick marks point downhill. Seamounts counted in this study are shown circled and shaded; unshaded seamounts do not meet our formal criteria, but are inferred from the morphology. The segment boundary marks where the volcanic ridge becomes discontinuous and offset.



seamounts with heights ≥ 200 m, conspicuous features on our multibeam data swaths, in the 17×10^3 km² of Sea Beam coverage outside the median valley floor in our survey. Although these values are about a quarter of the expected number, indicating the loss of many seamounts through faulting, the densities are still greater than other investigators have observed. Epp and Smoot¹⁵ reported no ridge-crest-generated seamounts ≥ 90 m high in the whole of the North Atlantic south of the Hayes fracture zone at $\sim 33^\circ$ N.

The magmatic style at the MAR, with volcanic ridges composed of abundant seamounts at the spreading axis, contrasts with that of the East Pacific Rise (EPR), where fissure-fed volcanism is dominant and small seamounts only start to appear outside the axial zone¹. The continuity of the magma chamber for tens of kilometres along the axis of the EPR¹⁶ indicates that on this scale the EPR behaves as a single elongated volcano erupting large volumes of magma through a fissure swarm analogous to the Hawaiian rift zones^{17,18}. Several studies suggest that large magma chambers are absent along the MAR^{19–22}. Eruptions from isolated pockets of magma would be both more localized and less copious. It is possible that the MAR seamounts represent the products of individual batches of magma rising from the mantle, evolving in small magma chambers and erupting from point sources. More probably they are produced from intermediate-sized magma chambers which are not large enough to sustain fissure eruptions but may feed several small volcanoes.

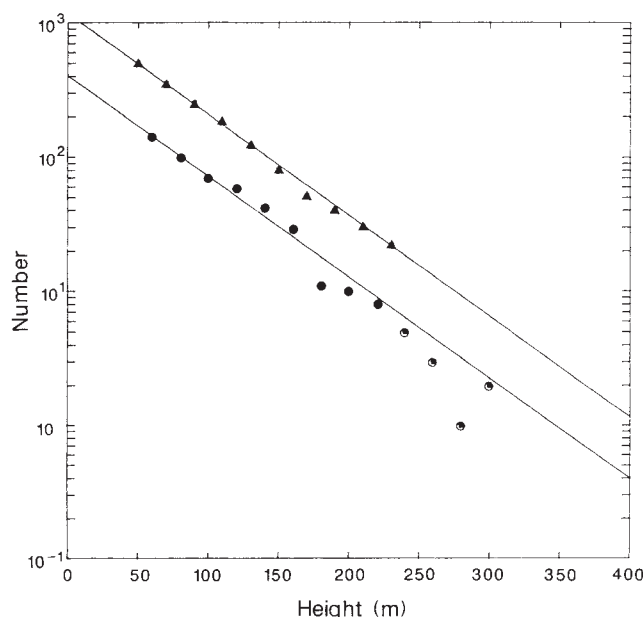


FIG. 3 Number of seamounts plotted against summit height. Circles are actual MAR seamount counts observed in 20-m height bins; triangles are the cumulative counts. The solid line is the maximum-likelihood fit^{5,6} to the binned data over the summit-height interval 50–210 m; half-open circles have been excluded from the fit.

In this region of the MAR we have identified a style of crustal construction unlike that of the well-known EPR. Here, and perhaps in many other intermediate- to slow-spreading ridges, small seamounts play a major part in crustal construction. This style is marked by axial volcanic ridges composed entirely of seamounts and yields seamount densities higher than observed elsewhere in the oceans. Point-source volcanism must lead to a structure for the extrusive section of the crust different from the fissure volcanism that dominates the EPR. Major seamount-dominated features may form a substantial part of the upper crust in a similar way to that suggested by Ballard and Van Andel²³. The overall effect would be very different from classic models of ocean-crust structure. □

Microbial control of silver mineralization at a sea-floor hydrothermal site on the northern Gorda Ridge

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THE Sea Cliff hydrothermal field, on the northern Gorda Ridge, contains mounds and chimneys of hydrothermally precipitated sulphide and sulphate minerals typical of sea-floor hydrothermal vent sites¹. In addition, large areas of the sea floor are covered by subhorizontal hydrothermal crusts. Samples of the crust recovered by submersible are composed of intensely altered fragments of basalt and basaltic hyaloclastite cemented by amorphous silica and chalcedony with less abundant barite, and minor amounts of base-metal sulphide minerals². Some surfaces of the crust were formerly colonized by bacterial mats, which are locally preserved by replacement and overgrowth of the bacterial filaments by metal sulphide minerals and amorphous silica. The bacterial filaments are selectively replaced by proustite (Ag_3AsS_3), pearceite³ ($\text{Ag}_{14.7-x}\text{Cu}_{1.3+x}\text{As}_2\text{S}_{11}$), chalcocite (Cu_2S) and rarely by galena (PbS). Our observations suggest that bacterially mediated processes selectively precipitate silver, arsenic and copper, and that biological processes may contribute to precious-metal enrichment in some sea-floor hydrothermal base-metal sulphide deposits.

High-temperature hydrothermal venting on the northern Gorda Ridge was detected by water-column chemistry and hydrographic surveys^{4,5}. The spatial distribution of chemical and temperature anomalies in the water column indicated that the site of high-temperature hydrothermal discharge was ~4 km east of, and 300 m shallower than, the spreading axis at 3,100 m depth. The structural setting and geology of the area under the hydrothermal plume have been investigated by geophysical surveys and bottom photography^{6,7}. Hydrothermal activity is

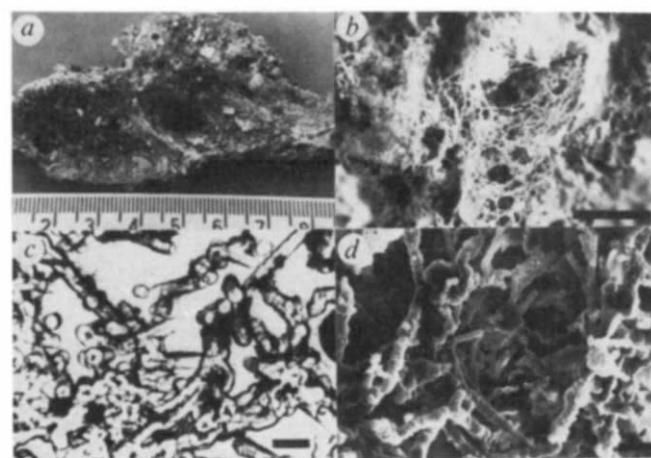


FIG. 1 a, Cross-section of a sample of hydrothermal crust. Most fragments are silicified basaltic glass. Largest fragment in centre is crystalline basalt altered to Mg-rich smectite and silica. (Scale divisions, 1 cm.) b, Silica encrusted bacterial mat on surface of hydrothermal crust. (Scale bar, 1 mm.) c, Bacteria replaced by sulphide (2-μm-thick dark filaments) overgrown by amorphous silica (50-μm-thick clear coatings). (Photomicrograph in transmitted light; scale bar, 100 μm.) d, Filamentous bacteria coated by amorphous silica. (Scanning electron photomicrograph; secondary-electron image; scale bar, 5 μm.)

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1. Fornari, D. J., Batiza, R. & Luckman, M. A. *Am. geophys. Un. Monogr.* **43**, 13–21 (1987).
2. Batiza, R. *et al. J. Geol.* **97**, 209–220 (1989).
3. Sempere, J.-C., Purdy, G. M. & Schouten, H. *Nature* **344**, 427–431 (1990).
4. Jordan, T. H., Menard, H. W. & Smith, D. K. *J. geophys. Res.* **88**, 10508–10518 (1983).
5. Smith, D. K. & Jordan, T. H. *Geophys. Res. Lett.* **14**, 1119–1122 (1987).
6. Smith, D. K. & Jordan, T. H. *J. geophys. Res.* **93**, 2899–2918 (1988).
7. Batiza, R. *Earth planet. Sci. Lett.* **60**, 195–206 (1982).
8. Kong, L. S., Detrick, R. S., Fox, P. J., Mayer, L. A. & Ryan, W. B. F. *Mar. geophys. Res.* **10**, 59–90 (1988).
9. Abers, G. A., Parsons, B. & Weissel, J. K. *Earth planet. Sci. Lett.* **87**, 137–151 (1988).
10. Vogt, P. R. *Earth planet. Sci. Lett.* **23**, 337–348 (1974).
11. Gorodnitskiy, A. M., Marova, N. A. & Sedov, A. P. *Dokl. Acad. Nauk SSSR, Earth Sci. Sect. (Engl. Transl.)* **243**, 1517–1520 (1978).
12. Litvin, V. M. & Rudenko, M. V. *Dokl. Acad. Nauk SSSR Earth Sci. Sect. (Engl. Transl.)* **213**, 944–947 (1972).
13. Kharin, G. S., Litvin, V. M. & Rudenko, M. V. *Volcanoes and Tectonosphere* (Tokai University Press, Tokyo, 1976).
14. Craig, C. H. & Sandwell, D. T. *J. geophys. Res.* **93**, 10408–10420 (1988).
15. Epp, D. & Smoot, N. C. *Nature* **337**, 254–257 (1989).
16. Detrick, R. S. *et al. Nature* **326**, 35–41 (1987).
17. Macdonald, K. C., Sempere, J.-C. & Fox, P. J. *J. geophys. Res.* **89**, 6049–6069 (1984).
18. Lonsdale, P. *Tectonophysics* **116**, 255–279 (1985).
19. Whitmarsh, R. B. *Nature* **246**, 297–299 (1973).
20. Fowler, C. M. & Keen, C. E. *Geophys. J. R. astr. Soc.* **56**, 219–226 (1979).
21. Purdy, G. M. & Detrick, R. S. *J. geophys. Res.* **91**, 3739–3762 (1986).
22. Detrick, R. S., Mutter, J. C., Buhl, P. & Kim, I. *Nature* **347**, 61–64 (1990).
23. Ballard, R. D. & Van Andel, T. H. *Geol. Soc. Am. Bull.* **88**, 507–530 (1977).
24. Smith, D. K. *Earth planet. Sci. Lett.* **90**, 457–466 (1988).

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TABLE 1 Representative electron-microprobe analyses of Ag-As-S minerals

Wt%	Proustite					Pearceite				
Ag	66.81	67.98	67.44	68.39	68.14	68.44	69.55	70.16	72.27	
S	18.63	18.77	18.14	18.15	18.14	15.85	16.35	16.41	16.17	
As	14.27	11.50	10.84	11.30	11.98	7.46	6.57	6.76	3.39	
Cu	0.06	0.21	0.18	0.18	0.18	5.76	5.81	5.66	5.33	
Fe	0.03	0.05	0.05	0.01	0.02	0.10	0.06	0.12	0.11	
Pb	0.07	0.95	0.91	0.48	0.32	0.04	0.00	0.52	0.16	
Sb	0.00	0.00	0.00	0.00	0.00	0.01	0.11	0.12	0.57	
Si	0.08	0.12	0.06	0.06	0.04	0.08	0.10	0.72	0.13	
Zn	0.07	0.24	0.88	0.01	0.07	0.64	0.58	0.53	1.91	
Total	100.02	99.82	98.50	98.58	98.89	98.38	99.13	101.00	100.04	

Standardized against synthetic sulphide and silver sulphosalt phases.

centred on the southern end of a 0.5-km-wide, 2-km-long linear ridge that is aligned parallel to the spreading axis. The west side of the ridge is bounded by a normal fault and is partly covered by basaltic talus. Hydrothermal activity seems to be confined to the intersection of a set of ridge-oblique normal faults and fissures, trending 350°, with the ridge-parallel normal fault that trends 022° (refs 6, 7).

The clear hydrothermal fluid issuing from chimney-like vent structures at temperatures up to 250 °C forms diffuse precipitates when the turbulent buoyant plume mixes with cold sea water. There are extensive areas of hydrothermal deposits and vent-specific faunal communities on the talus-covered slope of the ridge-bounding normal fault. Some areas of the sea floor are capped by a semicontinuous hydrothermal crust, which is locally inhabited by vent-specific fauna and bacterial-mat communities. Samples of this crust are composed of highly altered basaltic breccia and basaltic hyaloclastite cemented by amorphous silica² (Fig. 1a). Initial magnesian metasomatic alteration of the basaltic clasts forms saponite, which is replaced by amorphous silica and chalcedony as alteration progresses. The intense cation removal and silicification of basalt hyaloclastite is similar to the alteration observed in hydrothermal upflow zones underlying ophiolite-hosted massive sulphide deposits^{2,8}. The crusts also contain minor amounts of incorporated sediment, pelagic microfossils and 1–5 mm clasts of massive sulphide composed of pyrite, spalerite, chalcopryrite and trace amounts of galena. Proustite and pearceite have not been observed in the massive sulphide clasts. Barite is locally abundant in the matrix of the crusts; tabular void space overgrown by amorphous silica suggests the former presence of anhydride. Marcasite and pyrite, including pyrite framboids, are minor components of the siliceous matrix, but do not occur in the bacterial mats.

Submarine thermal vent sites are typically colonized by chemolithoautotrophic sulphur-oxidizing bacteria of the *Beggiatoa*, *Thiothrix* or *Thiovulum* type⁹, which are supported by chemical reactions with H₂S dissolved in the hydrothermal fluid. Live bacterial mats were observed at active vent sites in the Sea Cliff field. Samples of hydrothermal crust collected from the area surrounding the active vents contain fossilized communities of bacteria (Fig. 1b) which we interpret to have formed when hydrothermal fluid flowed through the crusts. Two morphologies of bacteria are observed: elongated filaments growing into open cavities (Fig. 1c), and dense clusters of shorter bacterial filaments, which form compact bacterial mats on the surface of the crust or lining open cavities. The size range and morphology of the fossilized bacteria are similar to 'flexibacteria' associated with *Beggiatoa* collected from active vent sites¹⁰. Fossilized filamentous bacteria have been described from ancient hydrothermal sites¹¹.

The bacteria are preserved by pseudomorphic replacement and overgrowth by sulphide and sulpharsenide minerals, and are coated by thick overgrowths of amorphous silica (Fig. 1c). The diameter of sulphide grains replacing bacterial filaments is typically between 1 and 2.5 µm. Some bacteria are not com-

pletely replaced by sulphide, and thin parallel-walled structures, ~1.5 µm wide and encapsulated in silica, extend from the termination of the sulphide grains. The sulphide-replaced filaments vary in length from a few micrometres to in excess of 200 µm. Many are straight rods of single sulphide crystals that connect with similar grains at angular intersections apparently representing the end of individual bacterial rods. Others have more curvilinear forms that occasionally bud and branch (Figs 1d, 2a) showing that sulphide minerals are pseudomorphically replacing the bacteria, not just nucleating on or in the bacteria.

The bacteria are selectively replaced by either chalcopryrite or silver sulpharsenide minerals. The fine grain size of the silver sulpharsenide phases, however, makes unambiguous mineral identification difficult (Fig. 2b). Examination by oil-immersion reflected-light microscopy shows that the grains have low reflectivity, low polishing hardness, anisotropy and deep-red internal reflections characteristic of the silver sulphosalt minerals. We analysed the grains using an electron microprobe. The results (Table 1) suggest that proustite and pearceite are the dominant minerals, and that these phases might be intergrown on a scale not resolvable by optical microscopy or electron-microprobe analysis. The minerals replacing the bacteria have a diameter equal to or slightly larger than the original bacterial filaments. The replaced bacteria also served as a nucleation site for the

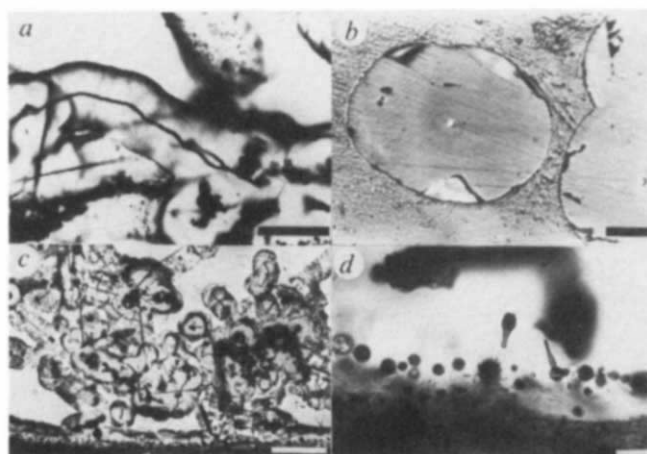


FIG. 2 a, Branching bacteria replaced by sulphide and overgrown by amorphous silica. (Photomicrograph in transmitted light; scale bar, 50 µm.) b, 2-µm grains of proustite (bright dots) replacing bacteria, overgrown by 50-µm-thick coatings of amorphous silica. (Photomicrograph in reflected light; scale bar, 10 µm.) c, Line of hydrocarbon fluid inclusions in amorphous silica along the boundary between dense bacterial mat (bottom) and bacterial filaments growing into an open cavity. (Photomicrograph in transmitted light; scale bar, 0.1 mm.) d, 2–4 µm hydrocarbon fluid inclusions, with vapour bubbles, encapsulated in amorphous silica. (Photomicrographs in transmitted light; scale bar, 10 µm.)

precipitation of other sulphide minerals that grew to coarser grain size. Minerals nucleated on replaced bacteria include sphalerite, galena, chalcopyrite and proustite.

The bacteria are uniformly overgrown by a coating 30–60 μm thick of amorphous silica with faintly visible concentric laminations. The silica encapsulation mimics the bacterial morphology (Fig. 1c), making the filamentous mats easily visible under low magnification (Fig. 1b). Similar coatings of amorphous silica overgrow and cement barite crystals and altered volcanic-rock fragments throughout the hydrothermal crusts.

The silica that encloses the bacterial filaments also contains two- and three-phase fluid inclusions. No fluid inclusions were observed in the ubiquitous amorphous silica matrix of the crusts away from encapsulated bacteria. Most inclusions are spherical and 1–3 μm in diameter (Fig. 2c, d). The inclusions are filled with a yellow liquid and contain vapour bubbles, which we estimate form 2–10 vol% of the inclusions. Some appear to have small, irregularly shaped, transparent solid inclusions. Larger ($\approx 15 \mu\text{m}$), irregularly shaped fluid inclusions contain similar ratios of yellow fluid and vapour, but do not have included solids. Some larger inclusions also contain a clear fluid phase, between the inclusion wall and the yellow fluid. We interpret the fluid inclusions to be spheres of yellow hydrocarbon, formed by thermogenic destruction of bacteria, and hydrothermal fluid trapped during deposition of amorphous silica. Liquid-hydrocarbon-bearing inclusions identical in appearance to those described above have been found in hydrothermal silica deposited at sea-floor hydrothermal sites in the Guaymas Basin¹².

The fossilized bacterial mats in the hydrothermal crusts are the site of silver mineralization in these samples, but the rock record cannot define the unique role of biochemical processes in concentrating silver. Bacterial mats have been observed to form on hydrothermal deposits through which there is a diffuse flow of low-temperature hydrothermal fluid ($<35^\circ\text{C}$)¹³. Enrichment of precious metals in submarine sulphide deposits also occurs in the outer, cooler ($<300^\circ\text{C}$) parts of the deposits owing to changes of fluid chemistry (such as f_{S_2} – f_{O_2} , where f is fugacity) induced by conductive cooling and mixing with cold sea water in the near-surface environment of hydrothermal vent discharge^{14,15}. The Sea Cliff bacterial mats may serve only as nucleation sites for precipitation of sulphides from hydrothermal fluids enriched in silver. Two observations suggest a more direct biogeochemical control on silver mineralization. First, silver minerals are only found in bacterial mats, and are not associated either with sulphide fragments in the crusts or with sulphide precipitated with the silica and barite that form the matrix of the crusts. Second, the bacterial filaments seem to be selectively replaced by chalcopyrite and proustite–pearceite, and are not observed to be replaced by associated sphalerite or by pyrite, even though sphalerite occurs as overgrowths nucleated on replaced bacteria and pyrite is the most abundant sulphide mineral in the crusts.

Experiments show that bacteria can efficiently remove silver from solution by concentration and precipitation on and within cell walls¹⁶. Biochemical concentration and segregation of elements such as Ag, As and Cu in bacterial organic matter may locally concentrate these metals to a level that exceeds the solubility of their sulphides, causing nucleation of chalcopyrite and proustite–pearceite and eventual replacement of the bacterial filaments by these minerals. Alternatively, the bacterial mat may influence physicochemical parameters to favour deposition of metal sulphide minerals from hydrothermal solution. For example, facultatively autotrophic, base-producing bacteria are common in enrichment cultures from hydrothermal vent sites⁹. A bacterially mediated increase of the pH in the micro-environment of bacterial mats may lead to deposition of metal sulphide minerals owing to decreased solubility at higher pH¹⁷.

We suggest the following sequence of events, all occurring within a few centimetres of the rock/sea water interface. Initial

low-temperature venting of hydrothermal fluid allowed colonization of bacterial mats on the surfaces of fragmented basalt in contact with oxygenated sea water. The bacteria concentrated Ag, As and Cu, eventually leading to the selective replacement of bacteria by chalcopyrite and proustite–pearceite. An apparent increase in fluid temperature, probably through decreased mixing of hydrothermal fluid with sea water owing to mineral precipitation, increased the precipitation of sulphide minerals, including some chalcopyrite, sphalerite and galena, which nucleated on the replaced bacteria and grew into the open space between bacterial filaments. This increased temperature also thermally degraded the bacterial mats, producing thermogenic hydrocarbon. Continued reduction of fluid flow allowed more conductive cooling of the fluid, resulting in extensive deposition of amorphous silica¹⁸, which trapped the hydrocarbon in fluid inclusions and encapsulated the bacterial mats and their sulpho-salt-replaced filaments. $\square$

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1. Rona, P. A. *et al.* *Geology* **18**, 493–496 (1990).
2. Schiffman, P., Zierenberg, R. A. & McClain, J. S. (abstr.) *Trans. Am. geophys. Un.* **70**, 1397 (1989).
3. Craig, J. R. & Scott, S. D. in *Sulfide Mineralogy* (ed. Ribbe, P. H.) CS1-CS110 (Miner. Soc. Am., Washington, DC, 1974).
4. Baker, E. T. *et al.* *Deep Sea Res.* **34**, 1461–1476 (1987).
5. Collier, R. W. & Baker, E. T. in *Gorda Ridge* (ed. McMurray, G. R.) 21–29 (Springer-Verlag, New York, 1990).
6. Rona, P. A. & Clague, D. A. *Geology* **17**, 1097–1101 (1989).
7. Clague, D. A. & Rona, P. A. in *Gorda Ridge* (ed. McMurray, G. R.) 31–50 (Springer-Verlag, New York, 1990).
8. Zierenberg, R. A. *et al.* *J. geophys. Res.* **93**, 4657–4674 (1988).
9. Jannasch, H. W. in *Hydrothermal Processes at Seafloor Spreading Centers* (eds Rona, P. A., Boström, K., Laubier, L. & Smith, K. L. Jr) 677–709 (Plenum, New York, 1983).
10. Nelson, D. C., Wirsén, C. O. & Jannasch, H. W. *Appl. envir. Microbiol.* **55**, 2909–2917 (1989).
11. Juniper, S. K. & Fouquet, Y. *Can. Miner.* **26**, 859–869 (1988).
12. Peter, J. M. *et al.* *Appl. Geochem.* **5**, 51–63 (1990).
13. Karl, D. M. *Biol. Soc. Wash. Bull.* **6**, 345–353 (1985).
14. Hannington, M. D. & Scott, S. D. *Econ. Geol. Monogr.* **6**, 491–507 (1989).
15. Large, R. R. *et al.* *Econ. Geol. Monogr.* **6**, 520–536 (1989).
16. Mullen, M. D. *et al.* *Appl. envir. Microbiol.* **55**, 3143–3149 (1989).
17. Garrels, R. M. & Christ, C. L. *Solutions, Minerals, and Equilibria* (Freeman, Cooper & Company, San Francisco, 1965).
18. Janeky, D. R. & Seyfried, W. E. Jr *Geochim. cosmochim. Acta* **48**, 2723–2738 (1984).

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Comet dust as a source of amino acids at the Cretaceous/Tertiary boundary

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LARGE amounts of apparently extraterrestrial amino acids have been detected recently in rocks at the Cretaceous/Tertiary (K/T) boundary at Stevns Klint, Denmark¹. The amino acids were found a few tens of centimetres above and below the boundary layer, but were absent in the boundary clay itself. If one supposes that these compounds were carried to the Earth by the giant meteorite thought to have impacted at the end of the Cretaceous^{2,3}, some puzzling questions are raised: why weren't the amino acids incinerated in the impact, and why are they not present in the boundary clay itself? Here we suggest that the amino acids were actually deposited with the dust from a giant comet trapped in the inner Solar System, a fragment of which comprised the K/T impactor. Amino acids or their precursors in the comet dust would have been swept up by the Earth both before and after the impact, but any conveyed by the impactor itself would have been destroyed. The observed amino acid layers would thus have been deposited without an impact.

Zhao and Bada¹ listed several observations in support of an extraterrestrial origin for the amino acids they detected. The two amino acids detected, α -aminoisobutyric acid (AIB) and isovaline, are rare in the biosphere but common in meteorites. The AIB to isovaline ratio is roughly the same at Stevns Klint as it is in meteorites. Neither is known to be an alteration product, and neither was detected in several other samples that they tested. Stevns Klint isovaline is racemic, as is generally the case for abiogenic amino acids but not for biogenic.

The AIB and isovaline were found tens of centimetres below and above the K/T boundary, but not farther from the boundary nor in the boundary clay itself (Fig. 1). By meteorite standards, the quantities of amino acid are large. Using 12 p.p.m. for AIB⁴ and 480 p.p.b. for iridium⁵, AIB/Ir in the Murchison carbonaceous chondrite is ~ 15 by mass. The total AIB content at Stevns Klint was inferred to be $\sim 5 \times 10^{-5} \text{ g cm}^{-2}$ (ref. 1) and the Ir content is $3.4 \times 10^{-7} \text{ g cm}^{-2}$ (ref. 6). The integrated AIB/Ir at the K/T boundary is therefore ~ 10 times that in the Murchison meteorite. Murchison is only a tenth as rich in carbon, hydrogen, oxygen and nitrogen (the 'CHON' elements) as small dust particles from comet Halley⁷. Although some or most of the Ir in the impactor could have escaped to space, depending on the impact velocity⁸, the integrated AIB to Ir ratio is nevertheless high, especially when one considers the fragility of a molecule of isovaline compared to that of an atom of iridium.

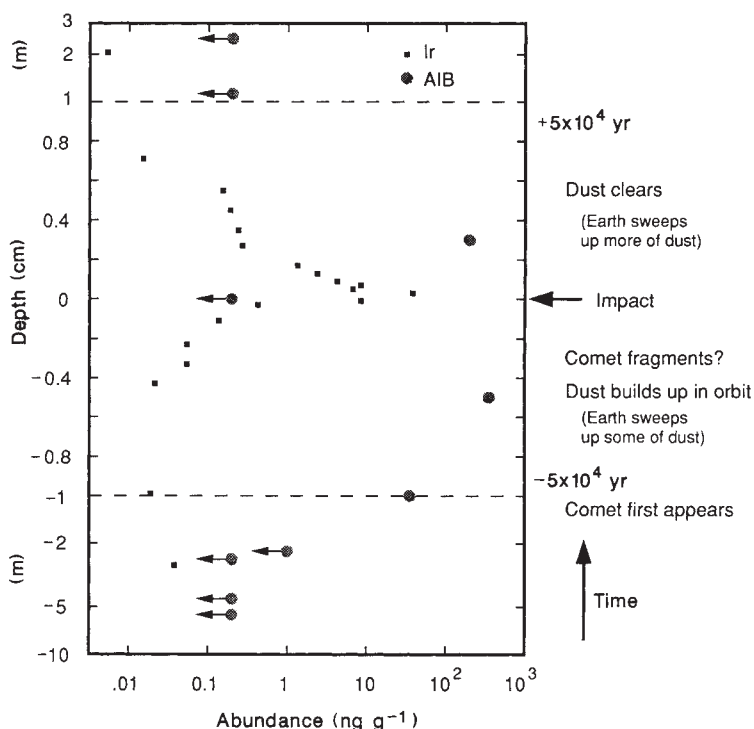
There are two objections to the hypothesis that the amino acids arrived in the impactor and later spread from the boundary clay to their present distribution. The first is that it is unlikely that a significant amount of amino acids would survive an impact of K/T scale, an impact that otherwise is only known to have been survived by elements like Ir, and for which other traces of surviving meteoritic matter (such as Xe still trapped in its high-temperature carrier) have been sought but not found^{3,9}. Incineration or atomization of the amino acids is more likely. The high temperatures and strongly oxidizing conditions in the fireball also argue against abiogenic synthesis of amino acids. The second objection is that diffusion from the boundary clay should not have left the boundary clay wholly depleted. This objection is applicable to any hypothesis that associates the amino acids with events involving the K/T impact directly.

An alternative to diffusion is to accept the sedimentary record at face value. We suggest that the extraterrestrial amino acids at Stevns Klint are cometary debris swept up by Earth, collected gently and non-destructively as interplanetary dust¹⁰. Particles smaller than $100 \mu\text{m}$ provide most of the exogenous organic material reaching Earth intact today³. Cometary dust is relatively rich in CHON elements (30%)⁷, and the particles are small enough that they would in general be expected to survive atmospheric entry intact. The comet need not have been amino acid rich, although it would support our model if it were. The interplanetary environment³ or the atmosphere¹¹ may have offered better environments for the synthesis of amino acids in dust grains than the comet. In any case, we require that comet dust contain either amino acids or their direct precursors, such as aldehydes or nitriles.

Reported deposition rates at Stevns Klint range from 1.9 cm kyr^{-1} (ref. 12) to 5 cm kyr^{-1} (above the boundary) and 12 cm kyr^{-1} (above and below the boundary, respectively)¹³. The slower rates are similar to those at other K/T boundary sites, such as Gubbio, Italy (1.1 cm kyr^{-1}) and Caravaca, Spain (3.6 cm kyr^{-1})¹². These rates are far from certain, but if they are accepted, the amino acids were deposited over 20,000 to 100,000 years. (If the amino acids were mobile, this is an upper limit.) This timescale is approximately the lifetime for evaporation of a big comet in a short-period orbit. Comet Halley, for example, is thought to be $\sim 3,000$ orbits old, having lost about 80% of its original mass¹⁴. A much larger comet could last 10^4 orbits or more. This corresponds to a lifetime of the order of 10^5 yr in a typical orbit for a short-period comet. Of course the lifetime could be shorter, especially if the comet were to disintegrate catastrophically.

Cometary evaporation could also explain the AIB profile. CHON-rich dust would have evolved from the comet over thousands of orbits before the impact. As the comet disintegrated the amount of dust in orbit increased, an increase reflected in enhanced AIB deposition. Dust remained in orbit after the impact, and so continued to be swept up for a few thousands or tens of thousands of years, a timescale consistent with its removal by Poynting-Robertson drag¹⁵. The lesser amount of AIB and isovaline above the boundary clay could be ascribed

FIG. 1 Possible interpretation of the sequence of events that occurred near the K/T boundary. Iridium⁶ and α -aminoisobutyric acid (AIB)¹ abundances near the K/T boundary at Stevns Klint, Denmark are plotted as a function of depth (mixed logarithmic and linear scales). Arrows denote upper limits. The timescale assumes a deposition rate of 1.9 cm kyr^{-1} (ref. 12).



to loss of the source. If the boundary clay was deposited quickly there would not have been time enough for it to collect much dust, which would account for the absence of amino acids. The base of the boundary clay is usually identified with direct fallout from the impact and probably was deposited quickly⁶. The bulk of the boundary clay (the 'fish clay'), however, was deposited more slowly. If deposition were slow enough, then some amino acid deposition would be expected. Arguments in favour of rapid deposition of the boundary clay are given by Kastner *et al.*⁶ and Gilmour and Anders¹⁶.

There are three difficulties with our hypothesis. The first, common to any putative extraterrestrial source, is that somehow AIB and isovaline must reach the sediments intact. Second, a high flux of comet dust is required. This difficulty is alleviated if the actual impactor were only a small remnant of an originally much larger object or one comet in a comet shower. Third, if the present AIB profile accurately records its deposition, AIB/Ir in the accreted dust must have been much higher than in meteorites. The resolution of this problem requires either an extremely AIB-rich source or segregation of Ir-bearing material from CHON-bearing dust.

Zhao and Bada¹ inferred average AIB abundances of 100 ng g⁻¹ in the sediment adjacent to the K/T boundary clay. The Ir concentration in the same sediments is ~0.02 ng g⁻¹ (ref. 6), and not obviously above background. If the Stevns Klint AIB profile accurately records the history of its deposition, AIB/Ir in our hypothetical comet dust must have been at least 5,000, which exceeds the ratio in the Murchison meteorite by 300. Either the dust was intrinsically AIB-rich or Ir-poor, or some combination of these. Taking AIB/Ir in the dust to be 300 times Murchison, and given a tenfold cometary enrichment in CHON elements and a tenfold Ir depletion (corresponding to AIB/CHON three times Murchison), for global coverage we would expect the accretion of ~7 × 10¹⁷ g of dust in 20,000 to 100,000 years. This corresponds to dust accretion rates of 2 × 10⁵–10⁶ g s⁻¹, roughly 10³–10⁴ times larger than today³.

It is reasonable to suppose that CHON-bearing dust is Ir-poor, as there is no reason to expect Ir to be found chiefly among the ices and organic molecules. It is conceivable that CHON dust was evolved or accreted preferentially. Segregation of rock and ice could be intrinsic to the comet, or a feature of cometary evolution, perhaps one peculiar to a large comet. For example, the comet may have accreted inhomogeneously, with ices precipitating on a rocky core. Iridium, a siderophile element, may have followed iron into a metallic phase that is under-represented in dust. Comets can lose volatiles preferentially; some, like Icarus or Phaethon, have evolved into asteroids¹⁷. Segregation could also stem from the relative density or strength of materials. Large meteorites (≥10⁵ g) cannot be accelerated to escape velocity from an ordinary comet by sublimating gases¹⁸. Current knowledge of the structure and composition of comets is clearly inadequate to rule out any of these possibilities.

Segregation could also be a product of orbital evolution. The much greater dust content in the inner Solar System caused by the evaporation of an enormous comet might produce a zodiacal dust regime quite different from today¹⁹. In particular, dust accretion would increase greatly if orbits were circularized by mutual collisions, thereby gravitationally enhancing Earth's collection efficiency. If CHON dust were smaller or more friable, it would be much more numerous and more subject to fragmentation and collisional circularization. To illustrate that mutual collisions can be important, we assume that Earth accreted spherical particles at the rate of $F \approx 2 \times 10^5 \text{ g s}^{-1}$. Then the mutual collision time between particles is $\tau \approx 4rp/3F$ where r and ρ are the radius and density of the particles. Taking $\rho = 1 \text{ g cm}^{-3}$, $\tau \approx 10^3 (r/100\mu) \text{ yr}$, which is shorter than the Poynting–Robertson timescale at 1 AU, ~7,000 $(r/100\mu) \text{ yr}$ (ref. 15). The total mass of a disk 1 AU in radius and 0.1 AU thick is ~10²⁰ g. If the orbits were circularized the required disk mass is reduced by the factor $(1 + v_{\text{esc}}^2/v_{\text{enc}}^2)$, where $v_{\text{esc}} = 11.2 \text{ km s}^{-1}$

is Earth's escape velocity and v_{enc} is the encounter velocity of the grains. This factor could be as large as 10.

Comets often split into pieces or otherwise disintegrate²⁰. Indeed, cometary breakup has been suggested as a way to get impact 'spikes' of 10⁴–10⁵ yr duration²¹. Extrapolation from the modern comet population implies that capture of a ~10²²-g comet into an inner Solar System orbit is a reasonably probable event over a 10⁸-yr timescale. As a very rough estimate, Encke, a comet with a 10⁴-yr lifetime, began as a ~3 × 10¹⁶ g object (Encke may be a fragment of a much larger object^{21,22}). For a cometary mass spectrum with $q \approx 1.7$ (ref. 23), a comet 5 × 10⁵ times more massive (~10²² g) might be expected over 10⁸ years. Chiron and the Great Comet of 1729 testify to the existence of comets in this general mass range. The average impact probability with Earth for a Jupiter-family short-period comet is ~1.1 × 10⁻⁹ yr⁻¹ (ref. 24). For Earth to accrete 7 × 10¹⁷ g of comet dust over 10⁵ years, the required source would need to have an initial mass of 6 × 10²¹ g. The collision of one 10¹⁸-g fragment of a 10²²-g parent with Earth is not unlikely. To illustrate, we divide the 10²²-g comet into 10⁴ equal pieces. For an impact probability of 1.1 × 10⁻⁹ yr⁻¹, 10⁴ fragments over 10⁵ yr would be expected to yield about one impact.

A comet shower, caused by a perturbation of the Oort cloud, has been suggested for the K/T boundary on other grounds²⁵, but works less well here, because a typical shower would have a timescale of ~0.8 Myr, a few times the orbital period of the dislodged comets²⁵. To reduce this to <10⁵ yr requires an encounter close enough to the Sun that most of the comets fall from inside 700 AU, a rare event expected on average only twice in the age of the Solar System²⁶. Such a shower would be extremely intense, exceeding the modern comet flux by a factor ~10⁵ (ref. 26). Olsson-Steel²⁴ conservatively estimates that long-period comets today produce one 10-km crater on Earth per 2.3 Myr. A flux 10⁵ times higher sustained for 10⁵ yr would leave 4,000 craters greater than 10 km and some 40 craters greater than 100 km. This is at least an order of magnitude too high for the K/T boundary. Because the intensity of the shower is proportional to the mass of the perturber, a brown dwarf or errant planet could in principle cause a comet shower short enough and sparse enough to match the record. If the 'missing mass' of the galactic disk is made of 0.08 $M_{\odot}$ brown dwarfs, then an encounter within 900 AU should occur in 330 Myr (ref. 27).

Our comet-dust model implies that, given equivalent preservation, the same temporal profile of AIB deposition should be seen globally. This can be tested at Stevns Klint, because the boundary clay is lenticular²⁸. A horizontally uniform distribution of AIB would support the comet-dust model, whereas a distribution correlating with the thickness of the boundary clay would support diffusion. A possible second detection of extraterrestrial amino acids at the K/T boundary has recently been reported for a site in Canada (D. Carlisle, personal communication). The amino acids at this site are reported to be located in the boundary clay itself and absent in the surrounding strata. If confirmed, this report would strengthen the case for diffusion at the Stevns Klint and would confirm amino acids as a widely dispersed phenomenon at the K/T boundary requiring non-destructive delivery. Our model also predicts that layers of extraterrestrial amino acids should be fairly frequent, although only rarely will the signal be as strong as at Stevns Klint. Because amino acid layers can form without an actual impact, these layers need not be correlated with other evidence for impact. If the dust shed by large comets frequently produced rich harvests of interesting chemicals on early Earth, and did so independently of whether the comet hit Earth, the potential for an important exogenic contribution to prebiotic organic chemistry is increased dramatically. □

Received 16 January; accepted 24 September 1990.

1. Zhao, M. & Bada, J. *Nature* **339**, 463–465 (1989).

2. Cronin, J. R. *Nature* **339**, 423–424 (1989).

3. Anders, E. *Nature* **342**, 255–257 (1989).
4. Cronin, J. R. & Pizzarello, S. *Adv. Space Res.* **3**, 5–18 (1983).
5. Anders, E. & Grevasse, N. *Geochim. cosmochim. Acta* **53**, 197–214 (1989).
6. Kastner, M., Asaro, F., Michel, H. V., Alvarez, W. & Alvarez, L. *Science* **226**, 137–143 (1984).
7. Jessberger, E. K., Kissel, J. & Rahe, J. in *Origin and Evolution of Planetary and Satellite Atmospheres* (eds Atreya, S., Pollack, J. B. & Matthews, M. S.) 167–191 (University of Arizona Press, 1989).
8. Vickery, A. & Melosh, H. J. in *Global Catastrophes* (eds Sharpson, V. & Ward, P.) (in the press).
9. Wolbach, W. S., Lewis, R. S. & Anders, E. *Science* **230**, 167–170 (1985).
10. Zahnle, K. & Grinspoon, D. *Bull. Am. astr. Soc.* **21**, 923 (1989).
11. Oberbeck, V., McKay, C. P., Scattergood, T., Carle, G. C. & Valentin, J. R. *Orig. Life* **19**, 39–55 (1988).
12. Preisinger, A. *et al. Nature* **322**, 794–799 (1986).
13. Bromley, R. G. in *Cretaceous-Tertiary Boundary Events* (eds Birkelund, T. & Bromley, R. G.) 16–32 (University of Copenhagen, 1979).
14. Hughes, D. W. *Astr. Astrophys.* **187**, 879–888 (1987).
15. Burns, J. A., Lamy, P. L. & Soter, S. *Icarus* **40**, 1–48 (1979).
16. Gilmour, I. & Anders, E. *Geochim. cosmochim. Acta* **53**, 503–511 (1989).
17. Olsson-Steel, D. *Icarus* **75**, 64–96 (1988).
18. Whipple, F. *Astrophys. J.* **113**, 464–473 (1951).
19. Whipple, F. *Mem. Soc. R. Liège, Ser. 6 LX*, 101–111 (1976).
20. Sekanina, Z. *Icarus* **58**, 81–100 (1984).
21. Clube, S. V. M. & Napier, W. M. in *The Galaxy and the Solar System* (eds Bahcall, J. & Matthews, M. S.) 260–285 (University of Arizona Press, 1986).
22. Stöhl, J. *Astr. Astrophys.* **187**, 933–934 (1987).
23. Donnison, J. R. *Astr. Astrophys.* **167**, 359–363 (1986).
24. Olsson-Steel, D. *Mon. Not. R. astr. Soc.* **227**, 501–524 (1987).
25. Hut, P. *et al. Nature* **329**, 118–126 (1987).
26. Hills, J. G. in *The Galaxy and the Solar System* (eds Bahcall, J. & Matthews, M. S.) 397–408 (University of Arizona Press, 1986).
27. Heisler, J. A., Tremaine, S. & Alcock, C. *Icarus* **70**, 269–288 (1987).
28. Surlyk, F. in *Cretaceous-Tertiary Boundary Events* (eds Birkelund, T. & Bromley, R. G.) 164–170 (University of Copenhagen, 1979).

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The role of disparity-sensitive cortical neurons in signalling the direction of self-motion

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MOVEMENT of an observer through the environment generates motion on the retina. This optic flow provides information about the direction of self-motion¹, but only if it contains differential motion of elements at different depths^{2,3}. If the observer tracks a stationary object while moving in a direction different from his line of sight, the images of objects in the foreground and in the background move in opposite directions. We have found neurons in the cerebral cortex of monkeys that prefer one direction of motion when the disparity of a stimulus corresponds to foreground motion and prefer the opposite direction when the disparity corresponds to background motion. We propose that these neurons contribute a signal about the direction of self-motion.

Consider an observer translating rightwards while tracking a stationary object in front of him (Fig. 1). Images of objects in the foreground (closer than the fixation point) will move to the left, while images of objects in the background (farther than the fixation point) will move to the right (Fig. 1). Without a signal indicating the depth of the two opposite motions, the direction of self-motion cannot be ascertained: the movement of the observer could equally well be to the left or the right. However, if the direction of flow could be tagged with a depth signal, the ambiguity about the direction of self-motion would be removed.

One depth signal is binocular horizontal disparity. In a frontal-eyed animal, the relative position of the images of an object on the two retinas indicates the depth of that object relative to the point of fixation. It has been proposed that the medial superior temporal area (MST) of the rhesus monkey cerebral cortex analyses optic flow^{4,5}, and we found in earlier experiments that direction-selective neurons in MST, the cells that respond to

fronto-parallel motion in one direction but not the other, carry a disparity signal. When presented with stimuli moving in their preferred direction, their discharge rate was higher when the stimuli were of one sign of disparity (for example, crossed disparities, foreground neurons) or the other (uncrossed disparities, background neurons) (J.-P.R. and R.H.W., manuscript in preparation). To test whether this disparity signal was appropriate to determine the direction of self-motion from the two opposite motions shown in Fig. 1, we presented a set of disparity stimuli moving first in the preferred and then in the non-preferred direction for the neuron under study.

We studied 65 cells. Their receptive fields were large (mean of 27° on a side, s.d. $\pm 12^\circ$) and often crossed the vertical meridian (57%). Of these 65 cells, 39 (60%) preferred the same sign of disparity for the two opposite directions of motion. In all these cells, however, one direction elicited a much stronger response to the preferred disparity than the opposite direction (Fig. 2a). These neurons could then detect the direction of motion of the foreground (as in Fig. 2a) or background. The relative depth signal added to the direction signal could provide information about the direction of self-motion. Under certain conditions, however, these neurons will fail to provide that information. If the observer looks at a very close object, for example, there will be only background motion, and a foreground responsive cell (such as the one in Fig. 2a) will be silent. These neurons then seem to signal the direction of self-motion only under certain conditions.

We found other neurons in MST that could play a more general part in signalling the direction of self-motion. Twenty-six cells out of the 65 tested (40%) preferred one direction of motion

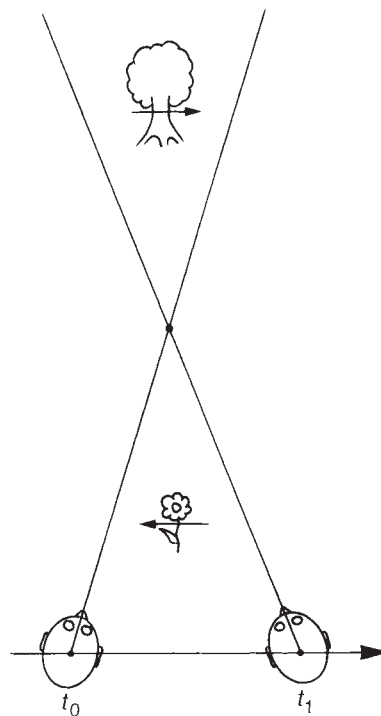
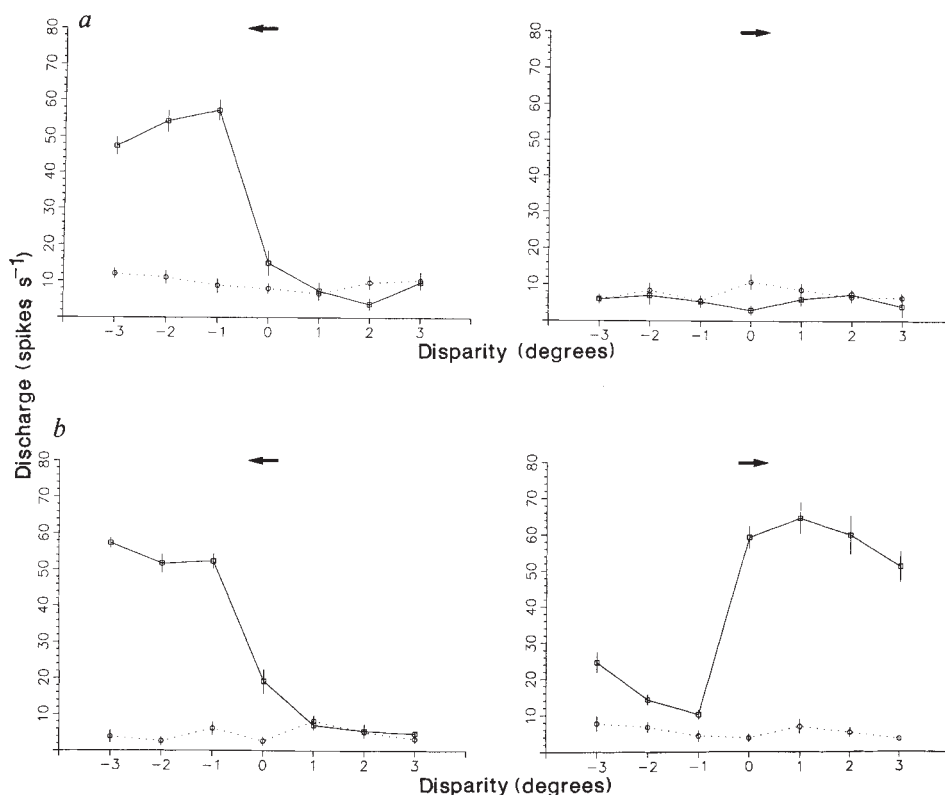


FIG. 1 Opposite motions of the background and foreground during self-motion (top view). As an observer moves to the right (large right-pointing arrow) while tracking an object, the direction of motion of the images of objects will depend on their depth relative to the point of fixation (the dot). An object in the background (behind the fixation point) 'moves' in the direction of self-motion; at time t_0 , the image of the tree is to the left of the line of gaze, at time t_1 , it is to the right: relative to the line of gaze, its image has moved to the right (small right-pointing arrow). An object in the foreground (in front of the fixation point) 'moves' in the opposite direction; at time t_0 , the image of the flower is to the right of the line of gaze, at time t_1 , it is to the left: its image has moved to the left (small left-pointing arrow). These opposite motions of the background-foreground will be generated when the observer does not look in the direction of his self-motion.

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FIG. 2 In *a*, an MST neuron discharges (continuous line) above the spontaneous rate (dotted line) for crossed disparities (indicated by a minus sign) when the stimuli move in one direction (left) but does not respond when the stimuli move in the opposite direction (right). Speed of motion for both directions is 6° s^{-1} . In *b*, another MST neuron discharges above the spontaneous rate for crossed disparities when the stimuli move in one direction (left), and discharges above the spontaneous rate for the opposite uncrossed disparities when the stimuli move in the opposite direction (right). Speed of motion for both directions is 6° s^{-1} . Random dot red/green anaglyph stimuli were presented at seven disparities: crossed: $-3^\circ, -2^\circ, -1^\circ, 0^\circ$; uncrossed: $1^\circ, 2^\circ, 3^\circ$. These stimuli were presented five times, randomly interleaved, in the central $20^\circ \times 20^\circ$ region of the neuron's receptive field. Receptive field size and eccentricity, preferred stimulus, preferred speed and preferred direction were determined, after which the disparity stimuli moving in the preferred and non-preferred direction were back-projected on a screen where the monkey fixated on a spot of light. The distance of fixation was maintained at 86 cm throughout the experiment. The position of both eyes was monitored using the search coil technique⁸ and the monkeys (two *Macaca mulatta*) had to maintain fixation within a $0.3^\circ \times 0.3^\circ$ electronic window throughout the stimulus presentation to receive a liquid reward. Single-cell discharges were counted in a time window of 400–1,000 ms after stimulus onset to



remove the non-directional on-response, and a time window of -600 – 0 ms was chosen for the spontaneous condition. Vertical lines indicate standard error over the five trials. Other experimental conditions were as before⁹.

when the monkey was presented with visual stimuli of one sign of disparity and the opposite direction of motion for visual stimuli of the opposite sign of disparity (Fig. 2*b*). This disparity-dependent direction selectivity means that the cell would respond during self-motion in one direction, irrespective of where in depth the animal is fixating. The neuron will respond when the foreground moves to the left or when the background moves to the right or both. These neurons then seem to signal the direction of self-motion relative to the object fixated under any conditions of viewing, as long as the direction of gaze and the direction of self-motion are different.

Most disparity-dependent directional selective neurons responded above the spontaneous level for one direction of motion when the disparity was 0° (as illustrated in Fig. 2*b* for rightward motion). This response could be associated with the foreground motion (4/20), background motion (9/20), both (5/20), or neither (2/20). This finding is consistent with the observation that rhesus monkeys are not able to track with perfect accuracy⁶. Their gaze either overcompensates for the self-motion causing the object tracked to 'move' with the background, or undercompensates causing the object tracked to 'move' with the foreground.

The relation between direction and disparity was not affected by varying the speed of motion of the visual stimuli: a disparity-dependent direction neuron did not become non-disparity-dependent when the stimuli moved at different speeds (6° s^{-1} , 16° s^{-1} and 26° s^{-1}). Also, the sign of disparity (crossed or uncrossed) for a given direction remained the same for all speeds tested. When other physiological properties of these neurons were compared, the only difference found was a predominance of disparity-dependent direction-selective neurons preferring horizontal motion (rightwards or leftwards) as opposed to oblique or vertical motion: 62% responded preferentially to a horizontal axis of motion, whereas only 36% of the non-disparity

dependent direction neurons responded preferentially to horizontal motion. This preference of disparity-dependent direction-selective neurons for horizontal motion supports our interpretation of a role for these cells in indicating the direction of self-motion: macaque monkeys are primarily terrestrial animals and so their locomotion will most often be horizontal⁷. Other physiological characteristics were equally distributed among the two types of neurons: preferred disparity, preferred direction, receptive field size, receptive field eccentricity, and frequency of receptive fields crossing the vertical meridian.

The disparity-dependent direction-selective neurons described here could provide a signal about the direction of self-motion when the observer moves in one direction while tracking an object in another direction. This corresponds to the condition where the direction of self-motion relative to the object tracked has a horizontal component. When there is no horizontal component to the self-motion, such as when the observer moves directly towards the object tracked, the optic flow is a pure expansion and the disparity-dependent direction-selective neurons, then, are unlikely to respond. Other neurons that do respond specifically to expansion have been described in MST^{4,5}. We propose that just as the expansion neurons could indicate the forward component of self-motion, the disparity-dependent direction-selective neurons could indicate the horizontal (rightward or leftward) component of self-motion. As the angle between gaze and self-motion changes during locomotion, these two cell types would provide a continuously changing signal about the direction of self-motion relative to the object tracked. □

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1. Gibson, J. J. *Perception of the Visual World* (Houghton Mifflin, Boston, 1950).
2. Rieger, J. & Lawton, D. *J. opt. Soc. Am.* **A2**, 354–360 (1985).
3. Warren, W. H. & Hannon, D. *J. Nature* **336**, 162–163 (1988).

4. Saito, H. *et al. J. Neurosci.* **6**, 145-157 (1986).
5. Tanaka, K. & Saito, H. *J. Neurophysiol.* **62**, 626-641 (1989).
6. Schwarz, U., Busetini, C. & Miles, F. A. *Science* **245**, 1394-1396 (1989).
7. Richard, A. F. *Primates in Nature* (Freeman, New York, 1985).
8. Robinsch, D. A. *IEEE Trans. Biomed. Electron.* **BME-10**, 137-145 (1963).
9. Komatsu, H. & Wurtz, R. H. *J. Neurophysiol.* **60**, 580-603 (1988).

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A human homologue of the yeast HDEL receptor

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RETENTION of resident proteins in the lumen of the endoplasmic reticulum is achieved in both yeast and animal cells by their continual retrieval from the *cis*-Golgi, or a pre-Golgi compartment¹⁻³. Sorting of these proteins is dependent on a C-terminal tetrapeptide signal, usually Lys-Asp-Glu-Leu (KDEL in the single letter code) in animal cells, His-Asp-Glu-Leu (HDEL) in *Saccharomyces cerevisiae*^{2,4,5}. There is evidence that the *ERD2* gene encodes the sorting receptor that recognizes HDEL in yeast^{6,7}; its product is an integral membrane protein of relative molecular mass 26,000 (26K) that is not glycosylated. In contrast, Vaux *et al.*⁸ suggest that the mammalian KDEL receptor is a 72K glycoprotein that they detected using an anti-idiotypic antibody approach. If this were so, it would indicate a surprising divergence of the retrieval machinery between yeast and animal cells. We report here that human cells express a protein similar in sequence, size and properties to the *ERD2* product, and propose that this protein is the human KDEL receptor.

We previously determined the sequence of the *ERD2* genes from two different yeasts, *Saccharomyces cerevisiae* and *Kluyveromyces lactis*^{6,7}. We prepared mixed populations of oligonucleotides corresponding to all possible codons encoding three conserved peptides (see Fig. 1). These oligonucleotides were used in the polymerase chain reaction (PCR)⁹ with complementary DNA prepared from a human T-cell line. PCR-generated clones were then used to screen a human placental cDNA library¹⁰. Two overlapping clones with inserts of roughly 1.2 and 1.7 kilobases (kb) were isolated; northern blots indicated a transcript of 1.7-1.8 kb. Sequence analysis revealed an open reading frame encoding a protein of 212 amino acids, with a 5' leader of about 350 bases that includes an in-frame termination codon 111 bases upstream of the initial ATG.

Figure 1 shows a comparison of the encoded human protein with the *S. cerevisiae* and *K. lactis* *ERD2* proteins. None of the proteins contain N-linked glycosylation sites. Overall, the three sequences show only 39% identity, but between either of the yeast proteins and the human one there is 50% identity, and 61% of the residues in the human sequence are found in at least one of the yeast proteins. So the divergence between the human and yeast proteins is not much greater than that between the two yeast receptors, which show only 59% identity.

The structural similarities are even more striking when conservative amino-acid changes are taken into account, as can be seen by comparison of the hydropathy plots of the human and *S. cerevisiae* proteins (Fig. 2). Seven distinct hydrophobic regions are found in each protein. Preliminary biochemical analysis of the yeast proteins has revealed no large luminal or cytoplasmic domain, suggesting that all seven segments may traverse the membrane. Charges within the hydrophobic sequences may form a binding site (for KDEL or HDEL) in a hydrophobic cleft or pocket, as is observed for several members of the seven-transmembrane-domain class of cell surface

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60
H M-NLFRFLGDLSHLLAIILLLLKIWSRSCAGISGKSVLFVAVFTARYLDLFT-NYISL
S M-NPFRILGDLSHLTSIILLIHNKTKTRYIEGISFKTQTLVAVFITRYLDLLTFHWVSL
K MLNVFRIAGDFSHLASIIILLIQSITTSNSVDGISLTKQLLYTLVFTITRYLNFT-KWTSL

120
H YNTCMKVVIYACSFITVWLIY-SKFKAT--Y-DGN-HDTFRVEFLVPTAILAFLVNHDF
S YNALMKIFFIVSTAYIVVLQGSKRNTTAYNEMLMHDTFKIQLHLLIGSALMSVFFHHKF
K YNFLMKIVFISSVYVIVLMRQKFKNPVAYQDMITRDQFKIFLIVPCILLGLIFNYRF

180
H TPLEILWTFISYLESVAILPQLFMVSKTGEAETITSHYLFALGVYRTLYLFNWIWRYHFE
S TFLLEWLSFVWLESVAILPQLYMLSKGGKTRSLTVHYIFAMGLYRALLYIPNWIWRYSTE
K SFIQICWSFSLWLESVAILPQLFMILTGTGKAKQLTSHYIFALGLYRALLYIPNWIWRYYTE
==1==>==2==>

221
H GF-FDLIAIVAGLVQTVLYCDDFFLYITKVLK-GKKLSLPA
S DKKLDIAFFAGLLQTLTLYSDFFIYYTKVIR-GKGFKLKP
K ER-FDKLSVFTGVIQTLVYSDDFFIYYQKVIKLGGLLELPQ
<==3==>

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FIG. 1 Comparison of the human (H), *S. cerevisiae* (S) and *K. lactis* (K) protein sequences (single-letter amino-acid code). Hyphens indicate gaps introduced to maximize homology (note that these are included in the numbering). Asterisks mark residues identical in all three proteins. The regions corresponding to the PCR primers are indicated by numbered arrows; primers 1 and 3, as well as 2 and 3, gave products whose sequence was identical to that of the cDNA clones. The human DNA sequence has been submitted to the EMBL database (accession number X55885).

receptors^{11,12}. If so, ligand binding and release may be accompanied by conformational changes that affect the entire receptor, which could in turn regulate its incorporation into transport vesicles.

The similarity of the yeast and human proteins encouraged us to attempt a functional assay of the latter in yeast, but expression plasmids containing the human sequence failed to complement the lethal phenotype of *erd2* deletion mutants and did not allow efficient retention of KDEL-tagged proteins in *S. cerevisiae* (data not shown). Attempts to express the *S. cerevisiae* protein in animal cells have also been unsuccessful.

To examine the intracellular distribution of the human protein we expressed an epitope-tagged version of it in COS cells (see legend to Fig. 3); tagging of the yeast receptor allows its detection with a monoclonal antibody but does not interfere with its function *in vivo*⁶. Immunoblotting indicated that the tagged protein had the expected gel mobility for a 25K polypeptide (data not shown). Staining of the transfected COS cells revealed high concentrations of the protein in a perinuclear structure (Fig. 3a) which was identified as the Golgi apparatus by double-labelling with an antibody specific for galactosyl transferase¹³ (Fig. 3b, c). In some cells there was also punctate staining over a wide area (Fig. 3d). This was distinct from the reticular pattern

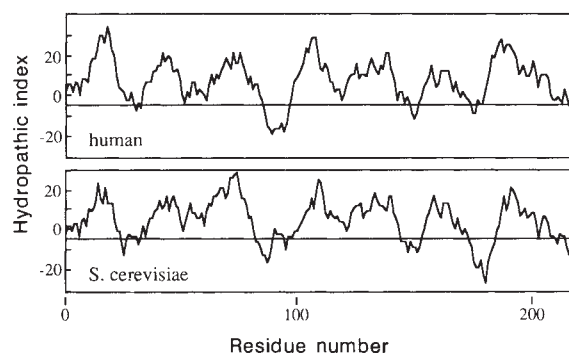


FIG. 2 Hydropathy plots of the human and *S. cerevisiae* proteins. The algorithm of Kyte and Doolittle¹⁶ was used, with a window of 11 amino acids. Regions above the horizontal lines have greater than average hydrophobicity.

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seen with endoplasmic reticulum (ER) markers⁴, but might correspond to transport vesicles, or to elements of the '15° compartment' that is thought to form a functional intermediate between ER and Golgi¹⁴.

Light microscopy cannot distinguish different cisternae of the Golgi complex, and caution must be used in interpreting the precise distribution of an epitope-tagged protein. Our results are consistent, however, with predictions that the KDEL receptor would be present in a post-ER compartment that may include tubulovesicular structures adjacent to the *cis*-Golgi and/or the first cisterna of the Golgi stack^{1,2,4,15}. The results are also in agreement with immunofluorescence and subcellular fractionation studies with yeast cells, which indicate that the receptor is concentrated in a post-ER compartment (ref. 6, and N. Dean, unpublished observations).

We believe that the human protein identified in this work is the KDEL receptor because it has a strikingly similar sequence, hydropathy profile and intracellular distribution to those of the yeast *ERD2* protein, and there is good genetic evidence that the latter is the HDEL receptor: *ERD2* controls both the capacity of the retention system and its signal specificity^{6,7}. Our protein is clearly distinct from the 72K glycoprotein described by Vaux *et al.*⁸ The suggestion that the 72K protein might be the KDEL receptor was based partly on the protein's ability to bind to two monoclonal antibodies whose variable regions are presumed to imitate a C-terminal KDEL sequence (because they can also

bind to anti-KDEL antibodies). In addition, the protein was located in the '15° compartment' and showed a weak affinity for KDEL peptides. These results are suggestive, and raise the possibility that human cells contain two dissimilar KDEL receptors. The genetics of *ERD2*, however, indicate that there is only one HDEL sorting receptor in *S. cerevisiae*.

In conclusion, our results show that the ER sorting receptor, like other components of the secretory machinery, has been strongly conserved during evolution. It can thus be studied using both yeast genetics and the biochemical methods developed for mammalian cells. □

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1. Pelham, H. R. B. *EMBO J.* **7**, 913-918 (1988).
2. Pelham, H. R. B. *A. Rev. Cell Biol.* **5**, 1-23 (1989).
3. Dean, N. & Pelham, H. R. B. *J. Cell Biol.* **111**, 369-377 (1990).
4. Munro, S. & Pelham, H. R. B. *Cell* **48**, 899-907 (1987).
5. Pelham, H. R. B., Hardwick, K. G. & Lewis, M. J. *EMBO J.* **7**, 1757-1762 (1988).
6. Semenza, J. C., Hardwick, K. G., Dean, N. & Pelham, H. R. B. *Cell* **61**, 1349-1357 (1990).
7. Lewis, M. J., Sweet, D. J. & Pelham, H. R. B. *Cell* **61**, 1359-1363 (1990).
8. Vaux, D., Tooze, J. & Fuller, S. *Nature* **345**, 495-502 (1990).
9. Saiki, R. K. *et al. Science* **239**, 487-491 (1988).
10. Simmons, D. & Seed, B. *Nature* **333**, 568-570 (1988).
11. Dohlmann, H. G., Caron, M. G., Strader, C. D., Amlaiki, N. & Lefkowitz, R. J. *Biochemistry* **27**, 1813-1817 (1988).
12. Dixon, R. A. F., Sigal, I. S. & Strader, C. D. *Cold Spring Harb. Symp. quant. Biol.* **53**, 487-497 (1988).
13. Roth, J. & Berger, E. G. *J. Cell Biol.* **93**, 223-229 (1990).
14. Saraste, J. & Kuismanen, E. *Cell* **38**, 535-549 (1984).
15. Warren, G. *Nature* **327**, 17-18 (1987).
16. Kyte, J. & Doolittle, R. F. *J. molec. Biol.* **157**, 105-132 (1982).

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Interconversion of CD45R subsets of CD4 T cells *in vivo*

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T LYMPHOCYTES express multiple forms of the leukocyte common antigen CD45, transcribed by alternative usage of leukocyte-common antigen exons 4-6 (refs 1-4). Species-specific monoclonal antibodies^{2,5-8} against restricted epitopes (CD45R) of the antigen subdivide CD4 T cells into reciprocal subsets expressing either the high molecular weight isoforms CD45RA or RB (ref. 4) or a molecule in which exons 4-6 have been spliced out (CD45R0)⁴. CD45R⁺ or RB⁺ CD4 T cells are potent in graft-versus-host reactions^{9,10} and interleukin-2 related activities^{5,6}, whereas the CD45R0⁺ subset responds *in vitro* to recall antigens^{5,11} and provides help for antibody synthesis^{5,9}. It is unclear whether CD45R subsets derive from separate lineages, or are products of unidirectional or reversible differentiation. We show by transferring CD45R⁺ or CD45R⁻ allotype-marked CD4 T cells into athymic nude rats that both subsets routinely generate cells of the opposite phenotype with a function that follows phenotype, not parentage. The recent equation of CD45R subsets as maturation stages representing 'naïve' and 'memory' T cells^{8,11-13} is difficult to reconcile with this finding.

Previous studies have established that PVG athymic nude rats became fully reconstituted with CD4 T cells following adoptive transfer of relatively small numbers of syngeneic thoracic duct lymphocytes (TDL)^{14,15} or of purified CD4⁺ TDL¹⁶. By transferring CD4 T cells from the congenic strain PVG-RT7^b (RT7b), donor-derived cells were subsequently identified by monoclonal antibody staining directed against the 'b' variant allotype¹⁵. During two years, the number of donor-derived CD4 T cells from surviving nudes increased many fold, displayed normal T-cell functions^{14,15} and maintained a stable antigen-reactive repertoire¹⁷. With this nude rat model we have investigated, both phenotypically and functionally, the progeny of CD45R

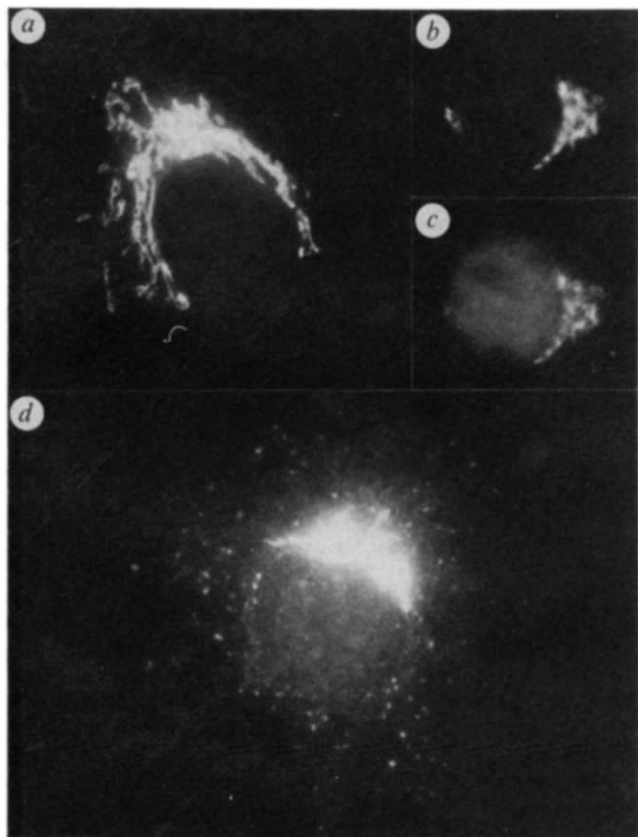


FIG. 3 Localization of the human *ERD2* homologue in COS cells. The cloned sequence was modified to encode the additional sequence TMEQKLISEEDLN (single-letter code) at the C terminus, inserted into a derivative of the expression vector SAGM2 (ref. 4) and transfected into COS cells. The expressed protein was detected by staining with monoclonal antibody 9E10 as previously described⁴. *a*, Typical Golgi-like staining pattern for the human protein. *b*, *c*, Double labelling of the expressed protein and galactosyl transferase (a *trans*-Golgi marker)¹³ respectively. *d*, A second type of staining seen in many of the transfected cells, with a dispersed punctate pattern in addition to the staining of the Golgi area.

TABLE 1 The expression of CD45R on CD4 T cells from peripheral blood or thoracic duct lymph 3 weeks (experiment 1) or 7.5 months (experiment 2) after transfer of purified CD45R⁺ or CD45R⁻ T cells to athymic nude rats

Experiment	Cells injected	<i>n</i>	Percent		45R ⁻	RT7b ⁺ (donor) and		Factor of donor cell expansion†	Per cent of donor cells positive for CD45R	
			TCR ⁺ and RT7a ⁺ (nude)	RT7a ⁻ (donor)		45R ⁺ dim*	45R ⁺ bright*		Before transfer	After transfer
1	1.6 × 10 ⁶ 45R ⁻	2	2.8	14.4	17.8	0.2	0.3	61	3.3	3.0
			3.1	13.3	16.8	0.3	0.3	58		
	3 × 10 ⁶ 45R ⁺	6	3.6	36.0	36.9	0.3	0.6	67.4	98.5	2.6
			±s.d.	±1.4	±11.0	±10.1	±0.2	±0.3	±17.5	±2.1
2	3 × 10 ⁶ 45R ⁻	2	ND‡	ND‡	22.8	4.8	7.7	82	0.3	38.0
					25.1	7.7	9.4	98		

PVG-rnu/rnu (RT7^a) nude rats were injected intravenously with purified subsets of TDL obtained from a congenic strain of allotype-marked PVG.RT7^b (RT7b) rats. RT7b TDL were depleted of surface immunoglobulin positive or B cells, CD8⁺ (experiment 1) and CD45R⁺ (experiment 2) cells by immunomagnetic separation after staining with saturating concentrations (1/10 ascites, monoclonals from Serotec Ltd, Oxford) of mouse anti-rat monoclonals OX12 (anti-immunoglobulin κ chain), OX6 (anti-MHC class II) OX8 (anti-CD8) (experiment 1) or OX6, OX8 and OX22 (anti-CD45R) (experiment 2). Antibody-coated cells were depleted first with anti-mouse immunoglobulin coated Biomag particles (Advanced Magnetics Ltd, Piddington) using 5 ml particle suspension per 10⁶ TDL and a second time using anti-mouse immunoglobulin coated Dynabeads (DynaL Ltd, New Ferry), 5 μ l bead suspension per 10⁶ cells, giving cells which were >98% CD4⁺ as determined by FITC-W3/25 (anti-CD4) staining. After immunomagnetic separation, cells for Experiment 1 were stained with FITC-OX22 and sorted into negative (channel 0–20) or positive (channel 58–255) fractions on either a FACS IV (Becton–Dickinson) or a Coulter Epics V flow cytometer. Cells for experiment 2 were stained with fluorescein isothiocyanate (FITC)-F(ab')₂-anti-mouse IgG (Dako Ltd, High Wycombe) and also purified by flow cytometry. Cell purities are recorded 'before transfer'. PBL (experiment 1) or TDL (experiment 2) were obtained from reconstituted nude rats 3 weeks or 7.5 months after transfer. Heparinized 1 ml blood samples were diluted in 3 ml PBS containing 1 U heparin ml⁻¹ and lymphocytes separated on Ficoll: Hypaque (63.7 ml of 14% Ficoll 400, Pharmacia; 20 ml 45% Hypaque, Sterling Winthrop). Cells (10⁴) were analysed on a FACScan using a Consort 30 program by two-colour fluorescence using a two-stage staining procedure: (1) 10 μ l mouse monoclonal (R73 (anti- $\alpha\beta$ T-cell receptor)¹⁸ or OX22 or W6/32 (control monoclonal, anti-human)) plus 10 μ l biotinylated rat monoclonal NDS58 (anti-RT7a, nude origin) or biotinylated 8G6.1 (anti-RT7b, donor); (2) 10 μ l 1/10 FITC-F(ab')₂-anti-mouse IgG plus 10 μ l 1/10 phycoerythrin-streptavidin (a gift from Dako Ltd).

* CD45R expression on T cells occurs in a continuous spectrum: negative cells were defined by control (W6/32) staining and the CD45R⁺ 'bright' population by OX22 staining of B cells. Between these two markers cells were scored as CD45R⁺ 'dim'.

† Observed number of donor cells in the recirculating pool divided by the number of donor cells injected. The size of the recirculating pool was previously determined¹⁴ in reconstituted nude rats at varying periods after TDL injection.

‡ ND, Not done.

CD4 T cell subsets (abbreviated 45R⁺ and 45R⁻) defined by antibody MRC OX22 (ref. 2) (OX22⁺ = CD45RB; OX22⁻ = CD45R0).

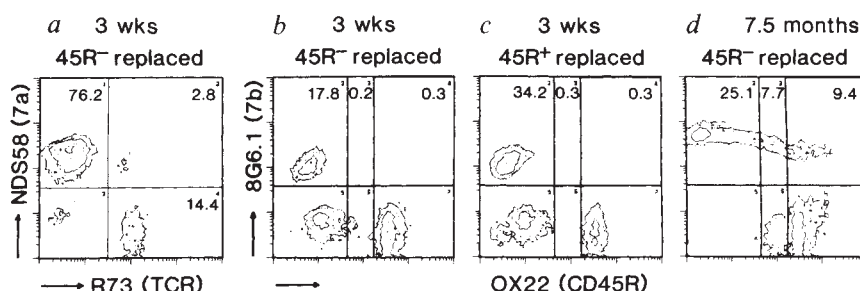
Three weeks after adoptive transfer, both 45R⁺ and 45R⁻ donor-derived T cells in peripheral blood showed a >60-fold expansion (Table 1). At this early stage, donor cells were essentially all 45R⁻ (Fig. 1b, c), irrespective of the phenotype of the injected population. TCR⁺ donor cells (RT7a⁺, Fig. 1a) could be clearly distinguished from TCR⁺, nonfunctional¹⁵ nude-derived cells (RT7a⁺). With time, 45R⁺ cells gradually appeared (S.M.S., E.B.B. and S. R. Sarawar, submitted), although even 6–8 weeks post-transfer, 45R⁺ offspring represented only 2–4% of the cells in lymph nodes, peripheral blood or thoracic duct lymph. At 7.5 months after the injection of highly purified 45R⁻ TDL, however, more than one third of donor-derived cells were 45R⁺ (Table 1, Fig. 1d).

Nude recipients of 45R⁻TDL were fully reconstituted and remained healthy for >2 yr, whereas nudes receiving 45R⁺ cells consistently failed, for reasons yet unknown, to survive beyond 2.5 months, despite a considerable expansion of CD4 donor T cells. As a consequence, analysis of 45R⁺ subpopulations was severely constrained. To ascertain the long-term fate of both CD4 T-cell subsets, nude rats were injected with a mixture of

purified 45R⁺ and 45R⁻ TDL bearing the RT7 a or b allotype as outlined in Fig. 2a. Twice as many 45R⁺ as 45R⁻ cells were transferred, reflecting the relative frequencies found in euthymic donors^{2,9,10}. Analysis of recipients' lymph nodes or TDL 2.5 to 7.5 months after injection showed that both 45R⁺ and 45R⁻ subpopulations expanded equally well, as in both combinations the original proportion of RT7a:RT7b donor-derived cells was maintained. Surprisingly, the CD45R subsets were interconvertible: 45R⁺ and 45R⁻ subsets of either RT7 allotype gave rise to both 45R⁺ and 45R⁻ progeny (Fig. 2b). Further experiments showed that these daughter cells had the functions of the new splice variant rather than that of the parental phenotype. 45R⁺ T cells are distinguished by their ability to give strong graft-versus-host responses⁹ in a popliteal lymph node assay¹⁴. We tested the 45R⁺ offspring of 45R⁻ parents as well as the reverse combination; in both cases, graft-versus-host activity reflected that of the new phenotype (Table 2). The switch in isoform was apparently linked with a physiological change within the cell.

It is currently accepted that the loss of CD45R reflects a unidirectional maturation from naive (45R⁺) to memory (45R⁻) T cells^{12,13}. We had therefore to consider whether the unexpected bidirectional switching of isoform was due to the preferential expansion of a small contaminating population.

FIG. 1 Analysis of donor-derived (RT7a⁺ or RT7b⁺) cells obtained from nude recipients reconstituted with CD45R-purified subsets of RT7b⁺ CD4⁺ TDL. Rats were injected with 45R⁻ (a, b, d) or 45R⁺ (c) subsets as described in Table 1. a, b, c, Analysis of PBL 3 weeks after injection; d, Analysis of TDL 7.5 months after injection. x-axis, green fluorescence (FITC); y-axis, red fluorescence (phycoerythrin).



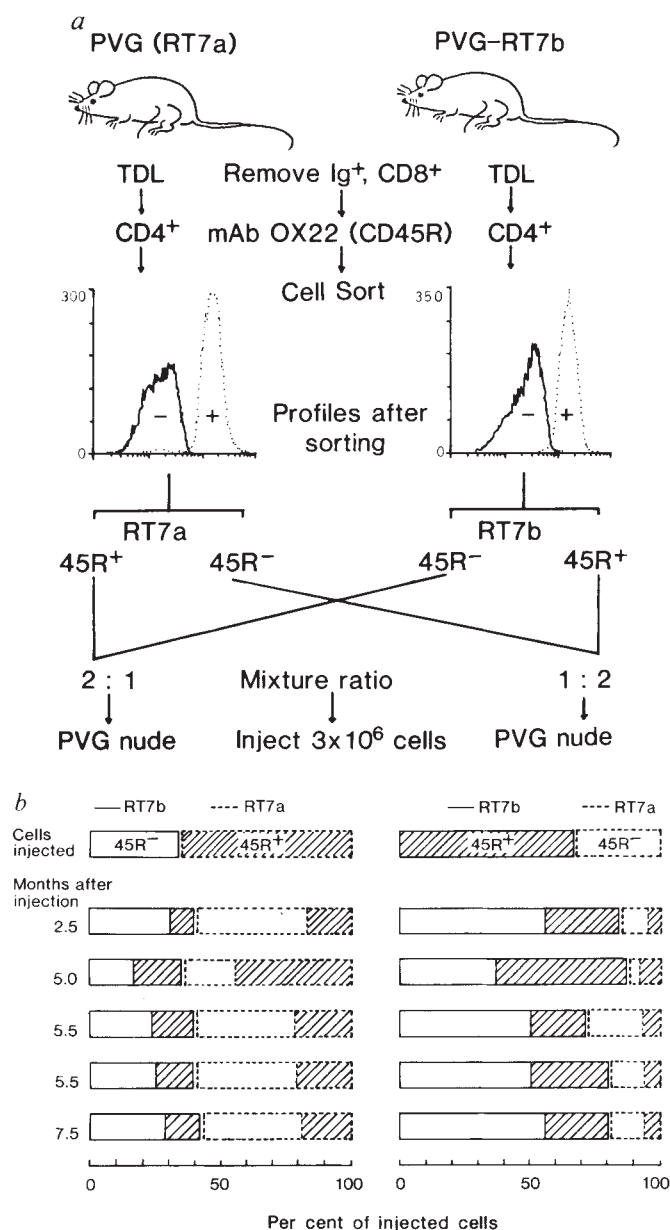


FIG. 2 Long-term developmental potential of CD45R CD4 T cell subsets following adoptive transfer to athymic nude rats. **a**, Experimental design. TDL from allotype-marked (RT7a or RT7b) donors were depleted of B cells and CD8 T cells, sorted into CD45R⁺ (45R⁺) and CD45R⁻ (45R⁻) fractions and recombined in the ratio of 2:1 (the proportion found in euthymic donors) for injection. The fluorescence profiles of the sorted populations are shown before cross-mixing. The purification procedure is described in Table 1; all four subpopulations were 97.0–99.6% pure before mixing. TDL or lymph-node cells were obtained from nude recipients 2.5 to 7.5 months later and dual stained for allotype (with monoclonals NDS58 or 8G6.1) and OX22, W3/25 or R73 (see Table 1 for details) or alternatively stained with biotinylated W3/25 and FITC-OX22. Populations depleted of B cells (with anti-rat immunoglobulin coated Dynabeads) were also analysed as nude B cells (RT7a⁺) also stain with OX22. Unseparated and B cell-depleted populations gave the same results; only the former are reported. **b**, Analysis of the CD45R phenotype of progeny of transferred, allotype-marked (RT7a, RT7b) 45R⁺ or 45R⁻ donor cells. Left, results from recipients of RT7a 45R⁺/RT7b 45R⁻ cells; right, results from recipients of RT7a 45R⁻/RT7b 45R⁺ cells. Broken lines, cells of RT7a origin; solid lines, cells of RT7b origin. Percentage of CD4⁺ (W3/25⁺) donor cells (RT7a+RT7b) ranges from 25.4 to 41.6% in individual recipients. For analysis, the total CD4⁺ donor population represents 100% and has been proportionally divided between RT7a and RT7b origin and each fraction subdivided according to the proportion expressing CD45R. The top bar illustrates the composition of the cells before injection. The other bars represent CD4⁺ donor cells, each from a single recipient; 10 reconstituted nude rats analysed.

TABLE 2 T-cell progeny of 45R⁺ or 45R⁻ donor T cells possess the GVH responsiveness of their new phenotype and not that of their parentage

Group	Cells originally injected*	Time after injection (months)	Phenotype of CD4 ⁺ progeny†	GVH‡ responsiveness R1 (mg)
a	45R ⁻	2	45R ⁻	13.11
b	45R ⁺	2	45R ⁻	12.51
c	45R ⁻	7.5	45R ⁻	11.17
			45R ⁺	62.95

* Purified CD4⁺ TDL sorted into 45R⁺ or 45R⁻ subsets before injection. Reconstituted nude donors comprising groups a and b in this table were from Table 1, experiment 1, but used 2 months post-injection. Group c reconstituted nude donors were the same as those in Table 1, experiment 2.

† Progeny of the originally injected cells were obtained as follows: TDL from reconstituted nude recipients were fractionated into CD45R subsets by immunomagnetic depletion (two cycles, see Table 1) after staining cells with OX6, OX8 and OX22 (Gp a, b) or OX12, OX6 and OX8 (Gp c). Pooled cells obtained from reconstituted nudes in Groups a and b (two in each group) were >98% 45R⁻ after purification. Pooled cells from two reconstituted nudes in group c (99.4% FITC-W3/25⁺) were stained with FITC-OX22 and cell sorted into 45R⁻ (99.7% pure) or 45R⁺ (99.9% pure) fractions.

‡ Purified CD4⁺ subsets (6.7 to 9.7 × 10⁵ cells in 0.1 ml PBS containing 2% fetal calf serum) were injected into footpads of (BN × PVG)_{F1} recipients using a standard graft-versus-host (GVH) assay¹⁴. Seven days later popliteal lymph nodes were removed, weighed and GVH activity expressed as R1 values¹⁴, that is, the calculated response (in mg of popliteal lymph node weight) induced by 10⁶ CD4⁺ T cells. Each R1 value was derived from the geometric mean weight of 4–6 popliteal lymph nodes. Control lymph-node weights (from footpads injected with PBS containing 2% FCS) were 4.95 mg (Gp a, b) and 6.30 mg (Gp c).

The experimental design shown in Fig. 2a minimizes this possibility. For each of the four subpopulations, what would, in one injected combination, be a rare contaminant would, in the reciprocal combination, become the majority phenotype. Any disproportionate increase in a particular subpopulation would be readily detected. The fact that allotype-marked cells increased equally in number regardless of their CD45R phenotype when injected (Fig. 2b), is incompatible with the selective or preferential expansion of rare contaminants. Furthermore, the observation that 45R⁺ cells gradually appear following an initial period when all transferred cells become 45R⁻ regardless of original phenotype (Table 1) is clear evidence of a reversible transition between 45R⁻ and 45R⁺ states.

It was also possible that 45R⁻ recent thymic migrants^{9,19}, which would theoretically copurify with putative 45R⁻ memory cells, were the source of the 45R⁺ progeny we observed. This seems unlikely; 45R⁻ progeny (several generations removed from the thymus) of 45R⁻ parental cells retransferred into secondary nude recipients also generated 45R⁺ offspring (S.M.S., E.B.B. and Sarawar, submitted manuscript).

Interconversion between 45R⁺ and 45R⁻ CD4 cells suggests that the concept that isoform expression reflects a unidirectional maturation from naive to memory function^{8,11–13} requires revision. The marker may rather reflect the physiological status of a T cell, in a functional division akin to the segregation of lymphokine production seen in murine helper T cell clones²⁰.

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- Sarmiento, M., LoKen, M. R., Trowbridge, I. S., Coffman, R. L. & Fitch, F. W. *J. Immun.* **128**, 1676–1684 (1982).
- Woollett, G. R., Barclay, A. N., Puklavac, M. & Williams, A. F. *Eur. J. Immun.* **15**, 168–173 (1985).
- Streuli, M., Hall, L. R., Saga, Y., Schlossman, S. F. & Saito, H. *J. exp. Med.* **166**, 1548–1566 (1987).
- Thomas, M. L. *An. Rev. Immun.* **7**, 339–369 (1989).
- Morimoto, C., Letvin, N. L., Distaso, J. A., Aldrich, W. R. & Schlossman, S. F. *J. Immun.* **134**, 1508–1515 (1985).
- Bottomly, K. *et al. Eur. J. Immun.* **19**, 617–623 (1989).
- Birkeland, M. L., Johnson, P., Trowbridge, I. S. & Pure, E. *Proc. natn. Acad. Sci. U.S.A.* **86**, 6734–6738 (1989).
- Terry, L. A., Brown, M. H. & Beverley, P. C. L. *Immunology* **64**, 331–336 (1988).
- Spickett, G. P., Brandon, M. R., Mason, D. W., Williams, A. F. & Woollett, G. R. *J. exp. Med.* **158**, 795–810 (1983).

10. Arthur, R. P. & Mason, D. *J. exp. Med.* **163**, 774–786 (1986).
11. Merkenschlager, M., Terry, L., Edwards, R. & Beverley, P. C. L. *Eur. J. Immun.* **18**, 1653–1661 (1988).
12. Sanders, M. E., Makgoba, M. W. & Shaw, S. *Immun. Today* **9**, 195–199 (1988).
13. Powrie, F. & Mason, D. *Immun. Today* **9**, 274–277 (1988).
14. Bell, E. B., Sparshott, S. M., Drayson, M. T. & Ford, W. L. *J. Immun.* **139**, 1379–1384 (1987).
15. Bell, E. B., Sparshott, S. M., Drayson, M. T. & Hunt, S. V. *Immunology* **68**, 547–556 (1989).
16. Whitby, E. H., Sparshott, S. M. & Bell, E. B. *Immunology* **69**, 78–84 (1990).
17. Drayson, M. T., Sparshott, S. M. & Bell, E. B. *J. exp. Med.* **170**, 691–702 (1989).
18. Hunig, T., Wallny, H. J., Hartley, J. K., Lawetzky, A. & Trefenhaler, G. *J. exp. Med.* **169**, 73–86 (1989).
19. Law, D. A., Spruyt, L. L., Paterson, D. J. & Williams, A. F. *Eur. J. Immun.* **19**, 2289–2295 (1989).
20. Mosmann, T. R. & Coffman, R. L. *Immun. Today* **8**, 223–227 (1987).

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Reduced levels of hsp90 compromise steroid receptor action *in vivo*

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SIGNALLING by steroid hormones is mediated by receptor proteins that bind hormonal ligands and regulate the transcription of specific genes. The heat-shock protein hsp90 seems to associate selectively with unliganded receptors (aporeceptors), but it has not been determined whether this interaction affects receptor function *in vivo*. To address the role of hsp90, we have taken advantage of the capacity of mammalian steroid receptors to function in yeast^{1–4}. We constructed a strain of *Saccharomyces cerevisiae* in which hsp90 expression was regulatable and could be reduced more than 20-fold relative to wild type. At low levels of hsp90, aporeceptors seem to be mostly hsp90-free, yet fail to enhance transcription; on hormone addition, the receptors are activated but with markedly reduced efficiency. Thus hsp90 does not inhibit receptor function solely by steric interference; rather, hsp90 seems to facilitate the subsequent response of the aporeceptor to the hormonal signal. This is the first biological evidence that hsp90 acts in the signal transduction pathway for steroid receptors.

S. cerevisiae carries two genes, *HSP82* and *HSC82*, that encode homologues of the mammalian hsp90 proteins. Mutants with defects in both genes are inviable, whereas either of the two genes alone is sufficient for growth at normal temperatures⁵. The yeast proteins share 60% identity with the human hsp90 protein⁶, and the mammalian homologue effectively complements the double mutant; furthermore, the glucocorticoid receptor functions equally well in yeast containing either one or two copies of the yeast gene or one copy of the human gene (data not shown).

We constructed a strain lacking both chromosomal genes and carrying a low-copy (centromeric) plasmid bearing *HSP82* expressed under the control of the galactose-inducible *GAL1* promoter. These cells die in glucose, but live in galactose media, in which hsp82 accumulates to a level around that of hsp82 plus hsc82 in the wild-type parent (data not shown). GRS4 is an isolate that is viable even in glucose; it produces ~5% of the wild-type level of protein in glucose, and the wild-type level in galactose. At 22 °C, the GRS4 strain grows at similar rates in glucose and galactose, but is strongly temperature-sensitive in glucose; all our experiments were carried out at ambient temperature (~22 °C).

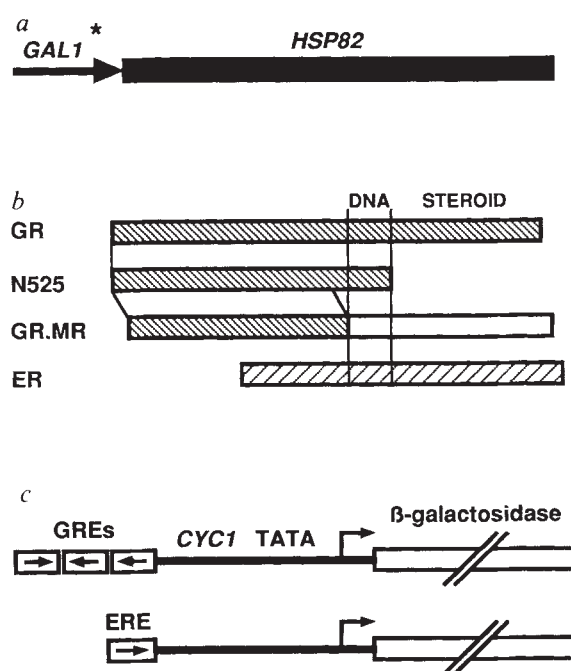


FIG. 1 Diagrams of DNA constructs. *a*, *S. cerevisiae* *HSP82* coding region expressed under the control of the *GAL1* promoter on a low copy number plasmid. The asterisk indicates leaky hsp82 expression under repressing conditions in glucose in strain GRS4, which allows normal growth at ambient temperature. *b*, Steroid receptors. Alignment is to the DNA-binding domains ('DNA'). All constructs were expressed from the glyceraldehyde-3-phosphate dehydrogenase promoter on a 2 μ plasmid. GR, rat glucocorticoid receptor (closely hatched bar); N525, constitutively active derivative of GR (amino acids 1–525); GR.MR, chimaeric rat mineralocorticoid receptor (open box) with sequences N-terminal of the DNA-binding domain replaced by those of GR; ER, human oestrogen receptor. *c*, Reporter plasmids. The *CYC1* TATA region fused at nucleotide position –178 either to three copies of a glucocorticoid response element (GRE) or to one copy of an oestrogen response element (ERE).

METHODS. The GRS4 strain: A diploid strain heterozygous for *Leu2*-marked disruption mutations in the *HSP82* and *HSC82* genes⁵ was transformed with a 2 μ vector carrying the *HSP82* and the *URA3* genes. The strain was sporulated and a haploid *hsp82 hsc82* strain (rescued by the plasmid) was selected. This haploid was transformed with a vector carrying the *TRP1* gene and a *Gal1 HSP82* fusion gene. The *Gal1 HSP82* fusion gene was created by inserting a *Bam*HI linker at a deletion endpoint 110 nucleotides upstream of the initiating ATG, converting the *Hind*III site in the 3' flanking region of the *HSP82* gene to a *Bam*HI site, and cloning the hsp82 coding sequences on the resultant *Bam*HI fragment downstream of the *GAL1* promoter of a pBM150 derivative²⁰ in which the *URA3* gene had been disrupted with *TRP1*. A strain lacking the wild-type *HSP82 URA3* plasmid was selected by plating cells on galactose with 5-fluoro-orotic acid. Finally, derivatives in which the *Gal1 HSP82* fusion gene was incompletely repressed by glucose were obtained by growth selection. p2HG (referred to as 'vector' in Figs 2 and 3), the expression vector for all steroid receptors, was obtained by cloning the *Eco*RI fragment from the 2 μ plasmid, and the *Eco*RI–*Bam*HI fragment from plasmid pGPD-2 (see ref. 1) containing the glyceraldehyde-3-phosphate dehydrogenase promoter, into plasmid pRS303²¹. Receptor complementary DNAs were cloned into the unique *Bam*HI site of p2HG. GR.MR, a *Bam*HI–*Xho*I fragment from plasmid pG-D²² encoding the GR amino-terminus (amino acids 1–414), was joined to sequences encoding the DNA- and hormone-binding domains of the rat MR (amino acids 604–981) generated as a *Sal*I–*Bgl*II fragment by the polymerase chain reaction (PCR) with a rat MR cDNA clone²³ as a template. ER sequences encoding the human oestrogen receptor (Gly 400 replaced by Val; refs 24 and 25), were inserted after introduction by PCR of a *Bam*HI site five nucleotides upstream of the translation initiation codon using the following oligonucleotide: 5'CGCGG-ATCCGGACCATGACC3'. The reporter plasmid with the GREs has been described before (pUC18 derivative of plasmid pSX26.1 (ref. 1); it contains three tandem copies of the tyrosine aminotransferase GRE (J. Thomas, unpublished results). The reporter plasmid with the ERE was obtained by inserting the synthetic oligonucleotide 5'TCGAGTCAGGTACAGTGACCTGAT-CAAAG3' (containing an ERE from the *Xenopus laevis* vitellogenin A2 gene²⁶) into the *Xho*I site of the pUC18 derivative of plasmid pSS (see ref. 1).

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Sequences encoding mammalian steroid receptors and receptor derivatives (Fig. 1) on 2μ expression vectors were introduced (together with appropriate reporter plasmids, see below) into the GRS4 strain. These included (1) the rat glucocorticoid receptor, GR; (2) a constitutively active GR derivative, N525; (3) a chimaeric rat mineralocorticoid receptor derivative, GR.MR, with sequences upstream of the DNA-binding domain replaced by those of GR (thus allowing detection with a GR-specific monoclonal antibody); (4) the human oestrogen receptor, ER. Results from immunoprecipitation and immunoblotting of extracts from these GRS4-derived strains revealed that receptor levels were similar (ER) or indistinguishable (GR and GR.MR) in glucose and galactose, whereas hsp82 levels were ~20-fold lower in glucose (Fig. 2).

To determine whether hsp82 associates with the receptor derivatives, the receptors were immunoprecipitated under non-denaturing conditions from cell extracts with GR- or ER-specific monoclonal antibodies; solubilized precipitates were elec-

trophoresed on SDS-polyacrylamide gels, blotted to nitrocellulose and probed with hsp82-specific antiserum, with appropriate receptor-specific monoclonal antibodies, or both. We found that the levels of co-precipitated hsp82 relative to immunoprecipitated receptors were substantially higher in galactose-grown cultures than in glucose (Fig. 2). Hsp82 production exceeded that of the receptors even in glucose (data not shown), implying that hsp82 interacts with other proteins as well as with receptors, and that free hsp82 is therefore limiting in glucose. With control strains containing expression vectors lacking receptor sequences, only very low levels of hsp82 coprecipitated under these same conditions. Thus, we conclude that aporeceptors indeed interact with the yeast hsp82, and that underexpression of hsp82 results in reduced levels of receptor-hsp82 complexes, with concomitant increases in hsp82-free receptor.

To assess receptor function, we monitored the expression of hormone-responsive reporter genes carried on 2μ plasmids. The TATA region of the yeast *CYC1* promoter driving the expression of β -galactosidase was linked to a cluster of glucocorticoid response elements (GRES) to measure activity of GR, N525 and GR.MR, or to an oestrogen response element (ERE) to measure ER activity. Hormonal ligands alone did not affect the expression of these reporter genes in the absence of coexpressed cognate receptors (ref. 4; and data not shown). As expected, the constitutive GR derivative N525 enhanced reporter gene expression equivalently in glucose and in galactose (Fig. 3a), demonstrating that transcriptional regulation *per se* by the receptor was unaffected by altering the carbon source and the hsp82 level. Furthermore, the functions of GR are not affected by such carbon source changes in the wild-type strain (data not shown).

It has been suggested that hsp90 may inhibit enhancement by blocking the DNA-binding domain of steroid receptors^{7,8}. We found, however, that apo-GR and apo-ER failed to enhance transcription when hsp82 production was curtailed in glucose, and only a small increase in basal activity was detected with apo-GR.MR under these conditions. Thus, hsp82 is not essential for suppression of the transcriptional enhancement activities of receptors in the absence of bound hormone.

In contrast, the hormonal activation of these receptors was strikingly dependent on the level of hsp82 expression. In the case of the glucocorticoid receptor, doses of various hormonal ligands that strongly activate receptor-mediated transcriptional enhancement under normal conditions produced little or no effect when hsp82 expression was repressed (Fig. 3a). In fact, only very high levels of the potent synthetic glucocorticoid deacetylcortivazol (DAC) produced a strong response in glucose. Parallel effects were observed with GR.MR and ER (Fig. 3b, c); that is, at relatively low hormone concentrations, receptor activities were substantially greater in galactose than in glucose, whereas at very high ligand concentrations, similar levels of enhancement were observed in galactose and glucose.

Our results indicate that aporeceptors produced under conditions in which they do not associate with hsp82 lack transcriptional enhancement activity, and that the ability of hormonal ligands to activate transcription functions is compromised. In contrast, others have shown that aporeceptors whose previous association with hsp90 (and perhaps with other factors) has been disrupted with high salt⁸ or temperature⁹ can display functional DNA binding and transcriptional regulatory activities¹⁰. Taken together, these results are consistent with the view that the hsp90-aporeceptor interaction both facilitates hormone binding¹¹ and affects receptor structure such that removal of hsp90 can result in the appearance of the other receptor activities (see below). Significantly, our results indicate that aporeceptor molecules that have never been complexed with hsp90 differ qualitatively from those whose previous hsp90 interaction has been reversed. This finding argues against the idea that hsp90 suppresses receptor functions merely by steric interference.

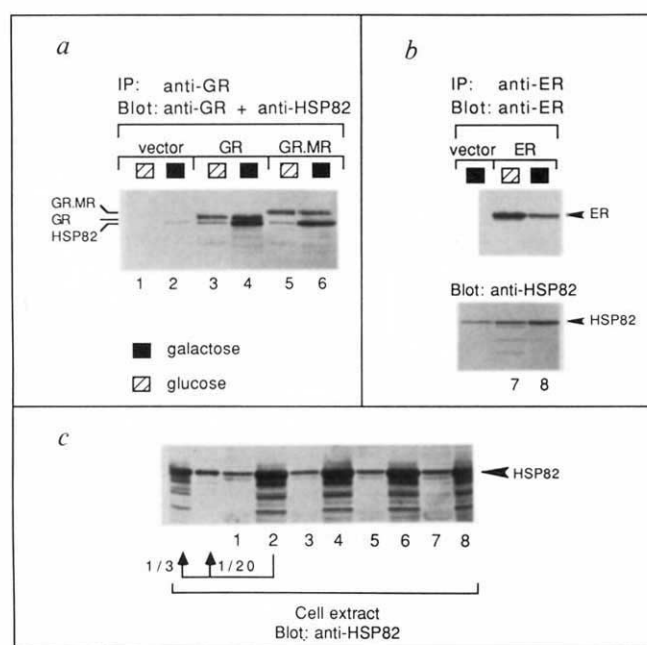


FIG. 2 Aporeceptors are predominantly hsp82-free at reduced levels of intracellular hsp82. Amounts of hsp82 complexed with GR and GR.MR (a) and ER (b) were assessed in immunoprecipitation ('IP') experiments. a, Yeast extracts were subjected to IP with monoclonal antibodies against GR and analysed by a western blot assay ('Blot'), probing with a monoclonal antibody specific for hsp82. b, After IP with a monoclonal antibody against ER, duplicate filters were probed either with the same antibody (top panel) or with an antiserum against hsp82 (bottom panel). c, Relative amounts of total hsp82 in extracts were determined by immunoblotting; numbers below the lanes identify the extracts used for corresponding experiments in a and b; extract 2 was diluted 1:3 and 1:20 for quantitation. 'Vector' indicates extracts from cells containing the expression vector without receptor sequences.

METHODS. Yeast cells were grown at ambient temperature in appropriate selection medium containing either 2% glucose or 2% galactose. Extracts were prepared at 4 °C by breaking cells with glass beads in 10 mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM EDTA, 1 mM DTT, 20 mM sodium molybdate, 15 mM MgCl₂, 20% glycerol, 1 mM PMSF, $\geq 1 \mu\text{g ml}^{-1}$ each of aprotinin, leupeptin, pepstatin A, and cleared at 60,000 r.p.m. for 30 min in a 70Ti rotor after a brief low speed spin. Equal amounts of protein were immunoprecipitated at 4 °C by adsorption to protein G-Sepharose (Pharmacia) in the buffer above supplemented with 0.2% Triton X-100; the precipitate was washed in the same buffer. GR, GR.MR, and ER were immunoprecipitated with mouse monoclonal BuGR2²⁷, mouse monoclonal 250²⁸, and rat monoclonal H222 (Abbott Laboratories), respectively. Immunoblots were probed with 250 (a), H222 (b top), and a rabbit antiserum against *S. cerevisiae* hsp82/hsc82 (a, b bottom and c) using appropriate secondary antibodies conjugated with alkaline phosphatase.

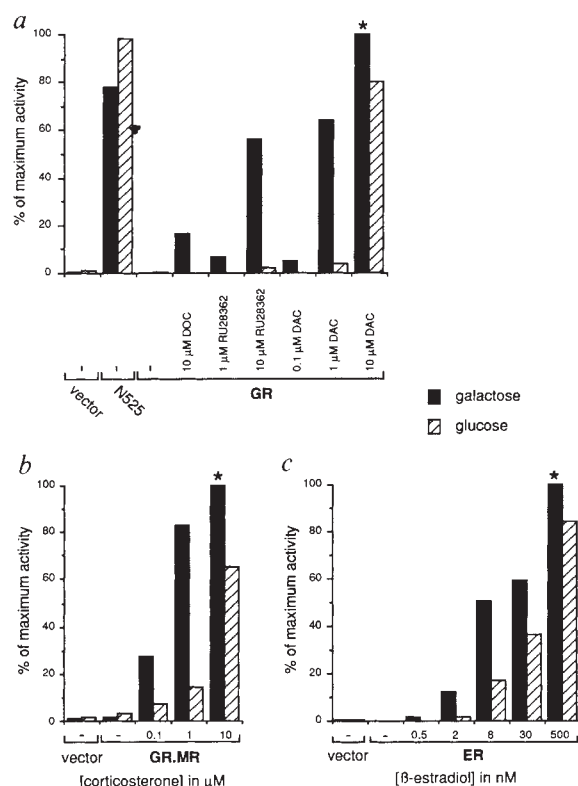


FIG. 3 Steroid receptor activities at normal and reduced levels of hsp82. β -Galactosidase activities from reporter plasmids are expressed as per cent of maximal induction observed for each panel (asterisk). Reporter plasmids contained GRs (a and b) or an ERE (c). The relative efficacies of particular ligands of the GR and GR.MR differ in yeast and mammalian cells (M.J.G. and D.P., unpublished results; see also ref. 4), therefore affecting the choice of the ligands shown (a and b). DOC, deoxycorticosterone; DAC, deacetylcortivazol (compound II in ref. 29). *S. cerevisiae* strain GRS4 was transformed with reporter and steroid receptor plasmids as indicated at the bottom of each panel. Cultures were grown at ambient temperature ($\sim 22^\circ\text{C}$), subcultured and treated with hormone overnight. β -galactosidase activities were determined as described³⁰ and corrected for culture density. The data represent average values from several experiments.

In previous studies, we showed that C-terminal receptor fragments encompassing the hormone-binding domains of apo-GR and apo-ER can inactivate various functions linked on the same polypeptide chain, even those of structurally distinct heterologous fusion proteins, and that this 'inactivation activity' is reversed on hormone binding and presumed hsp90 release¹²⁻¹⁴. Indeed, recent studies imply that hsp90 interacts with a discrete segment of the apo-GR C-terminal region¹⁵⁻¹⁷ and that deletions that produce a loss of inactivation activity¹⁸ correlate with the apparent hsp90 binding site¹⁷. On the basis of these results and on theoretical grounds, we proposed that hsp90 might have an active role in signal transduction by interacting with the aporeceptor and determining its ability to assume or maintain an activatable conformation¹⁹. Clearly, our present findings support that view. In particular, the scheme suggested that the hsp90 interaction would have two consequences: it would establish or stabilize the conformation of the active hormone-binding domain, and it would inactivate other functional domains in a manner that would readily be reversed upon hormone binding and hsp90 release. Those specific mechanistic predictions remain to be tested.

The differences that we have observed in steroid receptor activities at low and high intracellular hsp82 levels provide the first evidence that hsp90 participates *in vivo* in the mechanism of signal transduction by steroid receptors. Our results suggest either that receptors can be activated only if they are complexed with hsp82 (and therefore that more hormone is required to activate a sufficient number of receptors when aporeceptor-hsp82 complexes are limiting), or that hsp82-free aporeceptors can be activated but only at low efficiencies with high hormone concentrations. It should be possible in future studies to distinguish between these possibilities and to elucidate the nature of the apparent differences in the detailed behaviour of the different receptor species investigated here. In any case, we have identified an additional determinant in the signal transduction pathway for steroid hormones. Moreover, this approach may enable studies of more general features and biological functions of

hsp90, a family of highly conserved, strongly expressed genes present in all species from bacteria to mammals⁶. □

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- Schena, M. & Yamamoto, K. R. *Science* **241**, 965-967 (1988).
- Metzger, D., White, J. H. & Chambon, P. *Nature* **334**, 31-36 (1988).
- McDonnell, D. P., Pike, J. W., Drutz, D. J., Butt, T. R. & O'Malley, B. W. *Molec. Cell. Biol.* **9**, 3517-3523 (1989).
- Picard, D., Schena, M. & Yamamoto, K. R. *Gene* **86**, 257-261 (1990).
- Borkovich, K. A., Farrelly, F. W., Finkelstein, D. B., Taulien, J. & Lindquist, S. *Molec. cell. Biol.* **9**, 3919-3930 (1989).
- Lindquist, S. & Craig, E. A. *Rev. Genet.* **22**, 631-677 (1988).
- Groyer, A., Schweizer-Groyer, G., Cadepond, F., Mariller, M. & Baulieu, E.-E. *Nature* **328**, 624-626 (1987).
- Sanchez, E. R. *et al. J. biol. Chem.* **262**, 6986-6991 (1987).
- Willmann, T. & Beato, M. *Nature* **324**, 688-691 (1986).
- Klein-Hitpass, L. *et al. Cell* **60**, 247-257 (1990).
- Bresnick, E. H., Dalman, F. C., Sanchez, E. R. & Pratt, W. B. *J. biol. Chem.* **264**, 4992-4997 (1989).
- Picard, D., Salser, S. J. & Yamamoto, K. R. *Cell* **54**, 1073-1080 (1988).
- Picard, D., Kumar, V., Chambon, P. & Yamamoto, K. R. *Cell Reg.* **1**, 291-299 (1990).
- Eilers, M., Picard, D., Yamamoto, K. R. & Bishop, J. M. *Nature* **340**, 66-68 (1989).
- Pratt, W. B. *et al. J. biol. Chem.* **263**, 267-273 (1988).
- Dalman, F. C. *et al. J. biol. Chem.* **264**, 19815-19821 (1989).
- Howard, K. J., Holley, S. J., Yamamoto, K. R. & Distelhorst, C. W. *J. biol. Chem.* **265**, 11928-11930 (1990).
- Godowski, P. J., Rusconi, S., Miesfeld, R. & Yamamoto, K. R. *Nature* **325**, 365-368 (1987).
- Yamamoto, K. R., Godowski, P. J. & Picard, D. *Cold Spring Harbor Symp. quant. Biol.* **53**, 803-811 (1988).
- Johnston, M. & Davis, R. W. *Molec. cell. Biol.* **4**, 1440-1448 (1984).
- Sikorski, R. S. & Hieter, P. *Genetics* **122**, 19-27 (1989).
- Schena, M., Freedman, L. P. & Yamamoto, K. R. *Genes Dev.* **3**, 1590-1601 (1989).
- Patel, P. D., Sherman, T. G., Goldman, D. J. & Watson, S. J. *Molec. Endocr.* **3**, 1877-1885 (1989).
- Green, S. *et al. Nature* **320**, 134-139 (1986).
- Tora, L. *et al. EMBO J.* **8**, 1981-1986 (1989).
- Klein-Hitpass, L., Schorpp, M., Wagner, U. & Ryffel, G. U. *Cell* **46**, 1053-1061 (1986).
- Gametchu, B. & Harrison, R. W. *Endocrinology* **114**, 274-279 (1984).
- Okret, S., Wikström, A.-C., Wrangé, Ö., Andersson, B. & Gustafsson, J.-Å. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1609-1613 (1984).
- Simons, S. S. Jr, Thompson, E. B. & Johnson, D. F. *Biochem. biophys. Res. Commun.* **86**, 793-800 (1979).
- Yocum, R. R., Hanley, S., West, R. W. Jr & Ptashne, M. *Molec. cell. Biol.* **4**, 1985-1998 (1984).

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Limitation of the size of the vulval primordium of *Caenorhabditis elegans* by *lin-15* expression in surrounding hypodermis

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IN the nematode *Caenorhabditis elegans* six hypodermal cells, the vulval precursor cells, are each competent to generate vulval cells¹⁻². Normally only the three nearest precursor cells to the uterine anchor cell generate the vulva (22 nuclei), while the three others fuse with the non-specialized hypodermal syncytium (hyp7) surrounding each precursor cell and covering the body^{1,3}. Without an inductive signal from the anchor cell, all six vulval precursor cells fuse with hyp7 and no vulva is formed^{1,4}. But without activity of the vulval determination gene *lin-15(+)*, all six cells undergo vulval divisions whether the anchor cell is present or not⁵⁻⁸. Using mosaic analysis, we demonstrate here that *lin-15(+)* expression is necessary in cells other than the vulval precursor cells or the anchor cell, most probably in the hyp7 syncytium. We propose that *lin-15(+)* is active in hyp7 in order to repress an intrinsic vulval program in the precursor cells. The inductive signal from the anchor cell counteracts this repression for three precursor cells, allowing them to generate vulval cells (Fig. 1a). Such a two-signal (repressor/derepressor) mechanism may operate in other cases of tissue induction.

The 12 ventral hypodermal cells P(1-12).p are generated during the first larval stage³. In wild-type hermaphrodites, P(1-2).p and P(9-11).p fuse immediately with hyp7 and P12.p generates a unique, pre-rectal hypodermal cell, but P(3-8).p (the vulval precursor cells, or VPCs) remain uncommitted until the third larval stage, when the three VPCs nearest the anchor cell, P(5-7).p, commit to vulval development¹. Interactions among P(5-7).p and the anchor cell could determine the detailed fates of P(5-7).p (Fig. 1b; refs 8-10). The three remaining VPCs fuse with hyp7, usually after dividing once. Laser ablation of the anchor cell before VPC commitment causes all six VPCs to fuse with hyp7 (refs 1, 4).

In *lin-15* hermaphrodites, all six VPCs commit to producing descendants that form the vulva rather than fusing with hyp7 (Fig. 1c; refs 5-8). The enlarged vulval primordium typically generates two or three ventral protrusions (pseudovulvae) flanking a complete vulva; *lin-15(+)* is also required to prevent pseudovulvae development in males⁵. Laser ablation of the anchor cell in a *lin-15* hermaphrodite does not prevent the commitment of the six VPCs to vulval fates^{6,8}. These findings could be explained if *lin-15* acted only in the VPCs themselves, but our results contradict such a simple idea.

Genetic mosaics were generated by spontaneous loss of an X-chromosome fragment (free duplication) during embryonic divisions¹¹. The duplication carried *lin-15(+)*, and both complete X chromosomes carried a recessive *lin-15* mutation and cell-specific markers that allowed us to deduce where the duplication had been lost in the early embryonic cell lineage. Vulval development was normal in hermaphrodites with only a single copy of *lin-15(+)* carried by the duplication. Moreover, vulval development was fully mutant in those self progeny that failed to inherit the duplication; that is, any maternal *lin-15(+)* expression had no rescuing effect.

The lineal origins of the anchor cell, VPCs (P(3-8).p), and hyp7 are indicated in Fig. 2. The AB blastomere generates the VPCs; its sister P₁ generates the gonad, including the anchor

TABLE 1 Phenotypes of *lin-15* genetic mosaics

Point of duplication loss	Total No. of animals	No. of animals with given No. of pseudovulvae			
		0	1	2	≥3
P ₁ [†]	33	10	9	12	2
AB or AB.p [†]	13	0	0	7	6
AB.pl [‡]	10	4	0	4	2
AB.pr [‡]	13	5	2	5	1
P ₁ —no anchor§	16§	9§	3	2	2
Controls					
P ₀	50	0	0	0	50
No early loss¶	400	399	1	0	0
P ₀ —no anchor§	10	0	0	0	10
No early loss—no anchor§	40	40	0	0	0

* Animals carrying *unc-93(e1500) III; unc-3(e151) lin-15(n309) osm-1(p808) sup-10(mn219) X; mnDp14(X; f)[unc-3(+)/lin-15(+)] osm-1(+)* were screened for rare non-*Unc-93* non-*Unc-3* non-*Osm-1* hermaphrodites segregating only nullo-*mnDp14* self progeny (the germline descends from P₁), as described previously¹¹. *sup-10* is recessive suppressor of *unc-93(e1500)* (ref. 17); the *Unc-93* phenes—a characteristic uncoordination and a defect in egg-laying¹⁷—are suppressed when *sup-10(+)* is missing from body and egg-laying muscle cells as a result of duplication loss at P₁. The focus of *unc-3* action, which has a different effect on animal movement, is largely among descendants of AB.p (refs 11, 18). Certain amphid and phasmid neurons, which descend from AB.a, AB.pl and AB.pr, fail to incorporate fluorescein isothiocyanate when they do not have *osm-1(+)* (refs 11, 19-20). We sometimes used the lipophilic carbocyanine dye DiOC₁₈(3) (Molecular Probes) instead; living animals were exposed to 20 µg ml⁻¹ of DiOC₁₈(3) in M9 buffer¹⁹ for at least 2 h (D. Murray and E.M.H., unpublished results).

† Animals of genotype *unc-3 daf-6(e1377)lin-15 sup-10(n983); mnDp14* were screened for *Unc-3* non-*Sup-10* hermaphrodites, which were progeny-tested to show that they carried *mnDp14* in their germ lines. The gain-of-function mutation *sup-10(n983)* is distinct from the *sup-10* loss-of-function mutation; it confers a phenotype similar to *unc-93(e1500)* (ref. 21) and has its focus of action in muscle cells²². Several mosaics were also shown to be *Daf-6*, as judged by nonstaining of amphid and phasmid neurons²⁰, as described. The focus of *daf-6* action is in amphid and phasmid sheath cells^{18,23}, which derive from AB.pl and AB.pr.

‡ Animals of genotype *unc-3 daf-6 lin-15 sup-10(n983); mnDp14* were screened for semi-*Unc-3* non-*Sup-10* progeny, which were tested for staining of amphid and phasmid neurons. Duplication loss at AB.pl results in nonstaining of left amphid and left phasmid, and duplication loss at AB.pr results in nonstaining of right amphid and right phasmid¹⁸.

§ We used the *tra-1(rh132)* mutant, which has a male gonad and hermaphrodite soma, to eliminate the anchor cell. Because the gonad has no anchor cell, none of the VPCs is induced to vulval fates; all six usually divide once before fusing with hyp7. But because the VPCs are hermaphrodite, all six VPCs commit to vulval fates in double mutants with *lin-15*. The double mutants generally had 4-5 pseudovulvae but, as expected in the absence of an anchor cell, no normal vulva. The progeny of *unc-93 tra-1(rh132)/unc-93 dpy-18(e499); unc-3 lin-15 osm-1 sup-10; mnDp14* hermaphrodites were screened for *tra-1* homozygotes that had suffered duplication loss at P₁, using the same criteria as above except that, because the *tra-1* animals were sterile, we could not distinguish cases of duplication breakdown (*sup-10(+)* lost but *unc-3(+)* and *lin-15(+)* retained) from mosaics. In the experiments on the first line, these two classes were equally frequent (and the breakdown data were omitted). We therefore expect only half of the total animals scored here to be P₁(-) mosaics; the others should have no pseudovulvae. All 16 *tra-1* homozygotes were vulvaless. The control animals listed on the last two lines of the table were *Unc-3* (nullo-*mnDp14*) or *Unc-93* non-*Unc-3* (no early loss) homozygous *tra-1* progeny of the same stock used to generate P₁(-)—no-anchor mosaics.

|| These were nullo-*mnDp14* progeny of the hermaphrodite stock used to generate P₁(-) mosaics.

¶ 200 *Unc-93* non-*Unc-3* hermaphrodites of the same genotype as was used to generate P₁(-) mosaics and 200 non-*Unc-93* non-*Unc-3* hermaphrodites of the same genotype, except that their duplication did not carry *sup-10(+)*.

cell; both cells contribute to hyp7 (ref. 12). Thus an animal with duplication loss at P₁, designated a P₁(-) mosaic, carries a *lin-15(+)* gene in its VPCs but not in its anchor cell or in parts of hyp7. Most P₁(-) mosaics formed one or more pseudovulvae in addition to a complete vulva (Table 1). Vulval cell lineages for three such animals are shown in Fig. 3. The P₁(-) mosaics reveal that a *lin-15(+)* gene in P3.p, P4.p and P8.p is not in itself sufficient to ensure repression of vulval development in these cells; expression in one or more cells descended from P₁ is necessary. The P₁(-) mosaics did not display the fully mutant phenotype, however, indicating that *lin-15(+)* expression in AB descendants alone (such as the VPCs or hyp7) provides partial

repression. Indeed, AB(-) or AB.p(-) mosaics generally formed two or more pseudovulvae (Table 1), indicating that *lin-15(+)* expression in some AB descendants is necessary for fully normal repression.

In AB.pl(-) and AB.pr(-) mosaics, either P3.p or P4.p (but not both) is genotypically *lin-15* (Fig. 2). Some AB.pl(-) and AB.pr(-) mosaics formed pseudovulvae, but others underwent normal vulval development in that both P3.p and P4.p were repressed (Table 1). These animals indicate that a VPC that is itself *lin-15* can be repressed by *lin-15(+)* expression in other cells.

If the mutant genotype of the anchor cell in P₁(-) mosaics were responsible for pseudovulvae formation, animals without an anchor cell should develop identically whether or not they retain *lin-15(+)* in the P₁ blastomere. Our results proved otherwise: *lin-15(+)* animals lacking an anchor cell have no vulva or pseudovulvae, but P₁(-) mosaics lacking an anchor cell generally had 1-3 pseudovulvae (Table 1). Thus P₁ descendants other than the anchor cell contribute to vulval repression.

From a group of six equivalent VPCs, *lin-15(+)* activity limits the vulval primordium to the three cells induced by the anchor cell. The *lin-15* does not act cell autonomously: *lin-15(+)* VPCs can be induced inappropriately and *lin-15* mutant VPCs can be repressed correctly, depending on the genotypes of other cells. Unexpectedly, *lin-15* acts, at least in part, in cells other than the inducing or responding cells, namely the anchor cell and VPCs respectively. The focus of *lin-15* action in vulval development could not be narrowed by mosaic analysis; rather, descendants of AB.pl, AB.pr and P₁ seem to act cumulatively. All three cells make substantial contributions to hyp7 (Fig. 2). (By contrast, other tissue types, such as muscle, nerve or intestine, derive primarily from either P₁ or AB.) We propose that *lin-15(+)* and

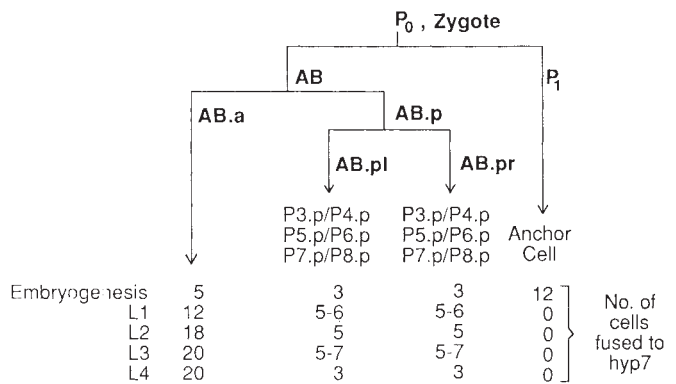


FIG. 2 Lineal origins of VPCs, the anchor cell of the gonad and the large hypodermal syncytium (hyp7). Only early embryonic divisions are shown¹². There are natural ambiguities in the lineal origins of the VPCs. For example, the distinction between cells P3 and P4 (mothers of P3.p and P4.p, respectively) is set by the order of the cells (P3 more anterior) after queuing along the ventral midline³. Hyp7 is formed during embryogenesis by the fusion of 23 cells, 11 derived from AB and 12 from P₁ (ref. 12). Additional cells, all descended from AB, are recruited during each subsequent larval stage.

possibly other multivulva genes^{5,7} are expressed in hypodermis (of both sexes), where they generate an intercellular signal that represses a vulval fate or promotes a hyp7 fate (perhaps by initiating cell fusion) in VPCs. The nature of the repressor signal and the role of *lin-15* in its generation are unknown. In the wild type, according to our model, the inductive signal from the hermaphrodite anchor cell acts by releasing the three VPCs nearest the anchor cell from repression by the hypodermis. In *lin-15* mosaics, the absence of the single copy of *lin-15(+)* from a significant fraction of hyp7 nuclei depletes expression within hyp7 to an extent such that additional VPCs can escape repression. (The syncytial nature of hyp7 may contribute to the variability observed in mosaic phenotypes; such variability has not been a feature of animal mosaics at other loci¹³.)

Previous models of VPC fate specification^{2,6,8,10} involved only the anchor cell and the VPCs. The involvement of hyp7 could mean, for example, that the anchor counteracts the repression of vulval development exerted by hyp7 by acting either directly

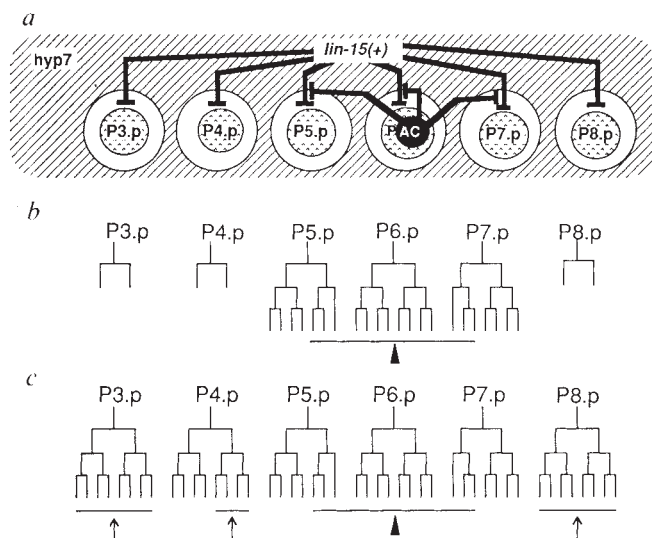


FIG. 1 Model of VPC induction and fates of VPCs. a, The drawing is a dorsal view of the ventral hypodermis²⁴; the anchor cell rests on the basement membrane of P6.p. A signal from hyp7 acts on the VPCs to repress vulval fates or promote non-vulval fates. This signal requires *lin-15(+)* expression in hyp7; the VPCs might also express *lin-15(+)*. The anchor cell (AC) of the uterus counteracts this repression for the three nearest VPCs, P(5-7).p, activating their intrinsic vulval programs. b, Divisions of VPCs in wild-type hermaphrodites. Each VPC expresses one of two vulval fates, called 1° (P6.p) and 2° (P5.p and P7.p), or a non-vulval fate, called 3° (P3.p, P4.p, P8.p). The latter cells fuse with hyp7, usually after dividing once. The choice between 1° and 2° vulval fates depends on interactions among committed VPCs⁸⁻¹⁰. The underlined cells detach from the cuticle and invaginate during morphogenesis of the vulva³. c, In *lin-15* hermaphrodites all six VPCs commit to 1° or 2° fates. The underlined cells in this example, scored during L3 lethargus, detached from the cuticle; each contiguous block of detached cells produced a vulva (filled arrowhead) or pseudovulva (arrows), externally visible after the L4 molt. The number of blocks of detached cells (and hence pseudovulvae) is slightly variable among animals.

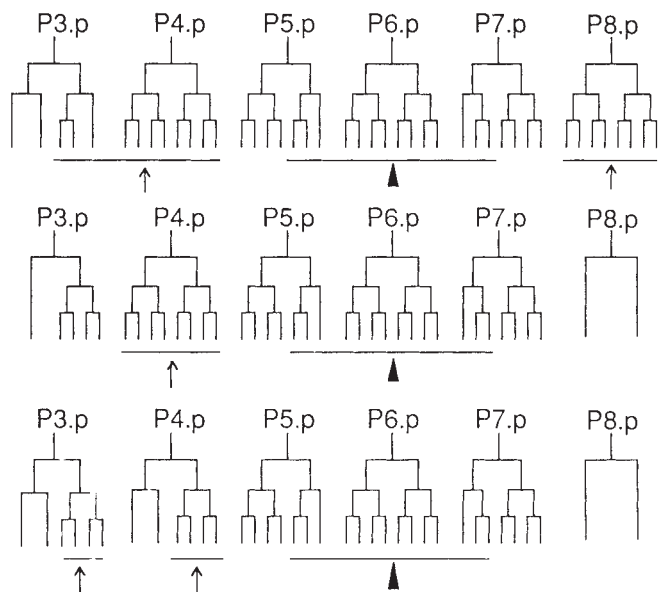


FIG. 3 VPC lineages in three P₁(-) mosaics. Each animal had a normal vulva (filled arrowhead) plus 1-2 pseudovulvae (arrows). A fourth P₁(-) mosaic (not shown) had wild-type lineages and no pseudovulvae.

on P(5-7).p or locally on the hyp7 nearest P(5-7).p; in the latter case there would be no direct communication between the anchor cell and the VPCs. Second, a role for hyp7 could explain why *lin-10* is expressed in cells other than the VPCs even though mutations in *lin-10* seem to affect only the VPC cell fates¹⁴.

A two-signal (repressor/derepressor) mechanism is common for hypodermal patterning in *C. elegans* and could underlie tissue induction in other phyla. For example, *lin-15(+)* seems to repress members of other equivalence groups besides the VPCs^{5,15}, although derepressors in these other cases are still unknown. Several lateral hypodermal cells (seam cells) are each competent to generate mating sensilla called rays; the ray fate is repressed by an unknown signal among hypodermal cells but derepressed by a signal, mediated by *pal-1(+)*, from the posteriormost seam cell¹⁶. Generally more cells are competent for induction than are actually committed to each hypodermal primordium. As repressor and derepressor signals are separately patterned, this mechanism may afford precise control of primordium shape. □

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1. Sulston, J. E. & White, J. G. *Dev. Biol.* **78**, 577-597 (1980).
2. Sternberg, P. & Horvitz, H. R. *Cell* **44**, 761-772 (1986).
3. Sulston, J. E. & Horvitz, H. R. *Dev. Biol.* **56**, 110-156 (1977).
4. Kimble, J. *Dev. Biol.* **87**, 286-300 (1981).
5. Ferguson, E. & Horvitz, H. R. *Genetics* **110**, 17-72 (1985).
6. Ferguson, E., Sternberg, P. W. & Horvitz, H. R. *Nature* **326**, 259-269 (1987).
7. Ferguson, E. & Horvitz, H. R. *Genetics* **123**, 109-121 (1989).
8. Sternberg, P. W. & Horvitz, H. R. *Cell* **58**, 679-693 (1989).
9. Greenwald, I. S., Sternberg, P. W. & Horvitz, H. R. *Cell* **34**, 435-444 (1983).
10. Sternberg, P. W. *Nature* **335**, 551-554 (1988).
11. Herman, R. K. *Genetics* **108**, 165-180 (1984).
12. Sulston, J. E., Schierenberg, E., White, J. G. & Thomson, J. N. *Dev. Biol.* **100**, 64-119 (1983).
13. Herman, R. K., *J. Neurogenet.* **5**, 1-24 (1989).
14. Kim, S. K. & Horvitz, H. R. *Genes Dev.* **4**, 357-371 (1990).
15. Fixsen, W., Sternberg, P., Ellis, H. & Horvitz, R. *Cold Spring Harb. Symp. quant. Biol.* **50**, 99-104 (1985).
16. Waring, D. A. & Kenyon, C. *Cell* **60**, 123-131 (1990).
17. Greenwald, I. S. & Horvitz, H. R. *Genetics* **96**, 147-164 (1980).
18. Herman, R. K. *Genetics* **116**, 377-388 (1987).
19. Hedgecock, E. M., Culotti, J. G., Thomson, J. N. & Perkins, L. A. *Dev. Biol.* **111**, 158-170 (1985).
20. Perkins, L. A., Hedgecock, E. M., Thomson, J. N. & Culotti, J. G. *Dev. Biol.* **117**, 456-487 (1986).
21. Greenwald, I. S. & Horvitz, H. R. *Genetics* **113**, 63-72 (1986).
22. Villeneuve, A. M. & Meyer, B. J. *Genetics* **124**, 91-114 (1990).
23. Albert, P., Brown, S. & Riddle, D. J. *comp. Neurol.* **198**, 436-451 (1981).
24. Kenyon, C. *Cell* **46**, 477-487 (1986).

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Gene replacement in parasitic protozoa

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TRYPANOSOMATID protozoa frequently cause severe diseases in humans. Many molecules likely to have a role during the infectious cycle have been identified, yet proof of their function is often lacking. We describe studies in *Leishmania major* of homologous gene targeting, a powerful method for testing gene function in other organisms¹⁻³. Following introduction of a construct containing dihydrofolate reductase-thymidylate synthase (*dhfr-ts*) flanking sequences fused to neomycin phosphotransferase, 45% of the colonies contained the planned homologous replacement; this frequency rose to nearly 100% in transfections using low amounts of DNA. Integrative transfection in *Leishmania* thus resembles that of *Saccharomyces cerevisiae* in giving predominantly homologous events. To facilitate studies of folate metabolism and

TABLE 1 Summary of transfection results

Experiment no.	Recipient cell*	DNA†	Amount (μg)	Total colonies	Pattern‡	
					Simple (replacement)	Complex (integrative)
1	CC-1	C	10	300	—	—
		L	10	152	0/8	8/8
2	CC-1	L	10	14	2/5	3/5
		L	5	7	7/7	0/7
3	CC-1	C	10	214	—	—
		L	3	13	2/7	5/7
		L	1.4	7	2/2	0/2
5	CBrev3	C	10	800	—	—
		L	2.3	70	2/2	0/2
		L	1.0	60	4/5	1/5
Fragment total:					17/36	19/36
'low DNA':					13/14	1/14

* CC-1 is a clonal derivative of the diploid LT252 line of *L. major*⁴; CBrev3 is the clonal heterozygous deletion line described previously⁹.

† C, Circular DNA control; pBgNEOA, a 9.4-kb *Bgl*II *neo* fragment (Fig. 2c) inserted into pBS-SK- (Stratagene); L, 8.3-kb *Kpn*I-*Bgl*II *neo* fragment from the vector depicted in Fig. 2c.

‡ Simple and complex patterns are defined in the text.

chemotherapy the sole *dhfr-ts* copy in a heterozygous deletion line was replaced, yielding lines that were functionally DHFR-TS⁻. Although most genes are diploid in trypanosomatids, methods exploiting the high frequency of homologous recombination should permit complete replacement of any parasite gene.

We showed previously that circular *dhfr-ts* plasmids containing the neomycin phosphotransferase gene (*neo*) in place of the *dhfr-ts* coding region can be efficiently introduced into *Leishmania* by electroporation, where they express chimaeric *trans*-spliced messenger RNAs and replicate autonomously as extrachromosomal elements^{4,5}. Work in other species⁶ suggested that linear DNAs would favour integration, although transfection of *Leishmania* with a fragment bearing complementary ends did not yield integrated DNA (ref. 7). We used a fragment with *neo* joined to 2.2 kilobases (kb) of 3' and 5.2 kb of 5' *dhfr-ts* flanking DNA, and non-complementary ends to reduce competing recircularizations (Fig. 2c). Colonies were obtained by plating on G418 selective media with nutritional supplements expected to permit growth of DHFR and TS auxotrophic mutants (legend to Fig. 1).

The *dhfr-ts* gene is normally located on the 500-kb chromosome V (ref. 8) and two classes of transfectants were observed: those retaining normal levels of chromosome V (45% of the lines examined, termed the simple class; Table 1; Fig. 1a, lanes 5, 6) and those exhibiting apparently reduced levels or even the absence of chromosome V (termed the complex class; Fig. 1a, lanes 1-3). In the simple class lines a *neo* hybridization probe identified a single chromosome indistinguishable from chromosome V, whereas one or more larger chromosomes were identified in the complex lines (Fig. 1b). The chromosomes that hybridized to a *neo* probe were shown to be linear by their behaviour when the pulse-time was varied⁸, although in the complex lines low levels of circular DNAs were also evident (not shown). The complex class arises from insertion of several copies of the transfecting DNAs into chromosome V, discussed below. Lines bearing exclusively circular *neo*-containing DNA were not observed.

The karyotype of the simple class colonies was that expected for a homologous gene replacement, and Southern blot analysis confirmed this view. Digestion with *Cla*I and *Spe*I revealed a 9.4-kb fragment in the wild-type DNA when hybridized with flanking probe A (Fig. 2a, lane 1), whereas the expected 2.9-kb replacement fragment was also observed in the simple class DNAs (Fig. 2a, lanes 2 and 3; Fig. 2c). Concordant results were obtained using flanking probe B, which tested the structure with a probe located at the other side of *neo* (Fig. 2b, c). These data indicated that the simple lines were heterozygous for the planned

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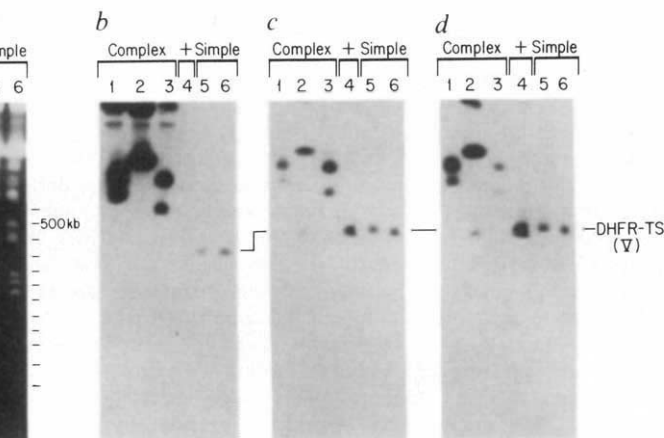
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FIG. 1 Molecular karyotype of *L. major* transfectants showing simple and complex transfectant classes. **a**, Ethidium bromide-stained gel. +, Diploid parental CC-1 line; 1, E1-4C5 (complex); 2, E1-4D2 (complex); 3, E2-6A3 (complex); 4, CC-1; 5, E2-7A1 (simple); 6, E2-7B2 (simple). E1- and E2- refer to the experiments listed in Table 1 using linear DNA. The position of the *dhfr-ts* chromosome V is shown. Marks on the right show the positions of λ concatamer markers. C, compression region. **b**, Hybridization of the blot of the gel shown in **a** with a *neo* probe (0.9-kb *SpeI* fragment from pSpeNEOA; ref. 4). **c**, Hybridization of the blot of a gel similar to that shown in **a** with a *dhfr-ts* probe (1.1 kb *BglII*-*EcoRV* fragment¹⁷). **d**, Hybridization of the blot shown in **c** with a probe specific for chromosome V flanking *dhfr-ts* (1.1 kb *EcoRI*-*EcoRV* fragment from phage LTS-14 flanking the amplified R region¹⁸).

METHODS. Cultured *L. major* line CC-1 was transfected by electroporation as described by Kapler *et al.*⁴ using 2.25 kV cm⁻¹ and 500 μ F in a BioRad Gene Pulser Apparatus. Cells were plated on semisolid M199 medium containing 16 μ g G418 per ml and KS supplements (10 μ g ml⁻¹ thymidine, 25 μ g ml⁻¹ methionine, 25 μ g ml⁻¹ glycine, 0.6 μ g ml⁻¹ bioprotein, 4 μ g ml⁻¹ folate, 50 μ M putrescine) and incubated

dhfr-ts replacement, and they retained a normally sized chromosome V hybridizing to *dhfr-ts* chromosome probes (Fig. 1c, d).

There was no detectable change in the nutritional requirements of the heterozygous replacement lines, an expected result, as a line heterozygous for a 30-kb deletion including the *dhfr-ts* gene showed no changes (CBrev3; see Fig. 3d)⁹. We then targeted the remaining copy of *dhfr-ts* in this line (Table 1). Pulsed-field electrophoresis again revealed two karyotypic classes: a simple class that showed a normal chromosome V hybridizing to the *neo* probe (Fig. 3a, b, lanes 5-7), and a complex class apparently lacking chromosome V and exhibiting *neo* hybridization to a single larger chromosome (not shown).



at 26 °C until colonies were evident. Colonies were transferred to liquid medium and grown for preparation of chromosome samples as described⁸. Pulsed field gels were run on a contour-clamped homogeneous electric field (CHEF) apparatus¹⁹ as described²⁰, with a pulse time of 55 s for 48 h. Blotting and hybridizations were as described⁴.

The karyotype of the simple class CBrev3 transfectants was that expected for homologous replacement of the remaining *dhfr-ts* copy: no hybridization was detected in these lines with a *dhfr-ts* probe (Fig. 3c, lanes 5-7; compare with lanes 1-4) although hybridization with a *dhfr-ts* flanking probe revealed the presence of both chromosome V homologues (Fig. 3d, lanes 5-7). Southern blot analysis confirmed that wild-type *dhfr-ts* fragments were absent, revealing only fragments corresponding to the planned alteration (Fig. 2a, b, lanes 5-7 versus lane 4; Fig. 2c).

These data indicated successful homologous replacement of the single copy of *dhfr-ts*. One metabolic consequence expected

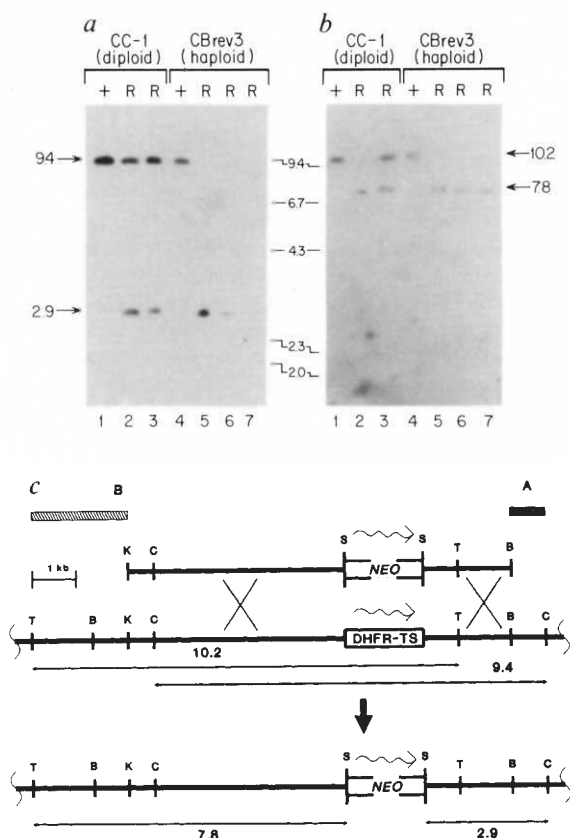


FIG. 2 Southern blot analysis of simple class transfectants. **a** and **b**, DNAs were digested with *Clal* and *SpeI* (**a**) or *SstI* and *SpeI* (**b**) and analysed by Southern blotting with the probes depicted in **c**; **a**, probe A; **b**, probe B. Plus sign indicates the parental and R the transfected lines, respectively. Molecular weight markers are shown (kb); the wild-type fragments were 9.4 and 10.2 kb, the replacement fragments 2.9 and 7.8 kb. Samples are 1, CC-1 (diploid); 2, E2-7'A3 and 3, E2-7'C3—both derived from CC-1; 4, CBrev3 (deletion heterozygote); 5, E5-5C1, 6, E5-6C5 and 7, E5-6'B6—all derived from CBrev3. All transfected lines received the *Kpn*-*BglII* fragment shown in **c**. **c**, Map of *dhfr-ts* locus, planned homologous replacement events, transfecting DNA fragments and hybridization probes. The top map shows the structure of the linear 8.2 kb *KpnI*-*BglII* fragment used for transfections, the middle map the *dhfr-ts* locus on chromosome V, and the bottom map the planned homologous replacement of *dhfr-ts* with *neo*. Relevant restriction sites (K, *KpnI*; B, *BglII*; T, *SstI*; C, *Clal*; S, *SpeI*), hybridization probes (stippled boxes) and predicted fragments in Southern blots are indicated. The *dhfr-ts* and *neo* boxes show the locations of the coding regions of those genes; dotted lines around *neo* signify that it is 0.8 kb shorter than *dhfr-ts*. Sizes (kb) are indicated.

METHODS. Chromosomes in agarose plugs were washed and digested with the indicated restriction enzymes and analysed by Southern blotting as described⁴ with the probes indicated.

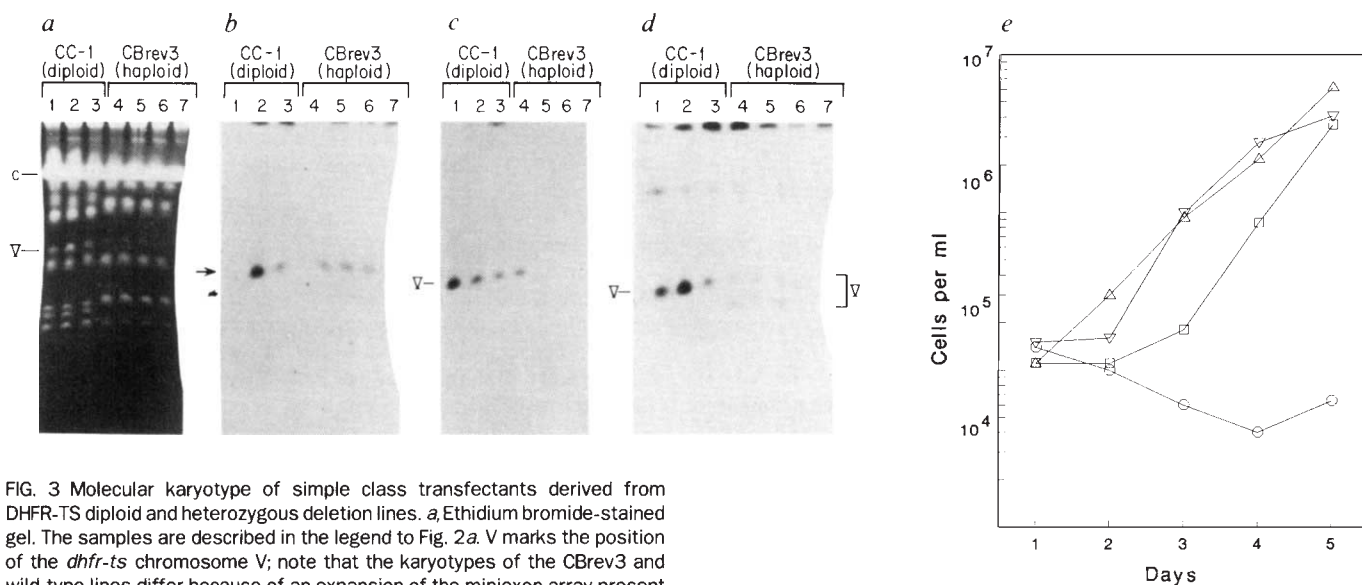


FIG. 3 Molecular karyotype of simple class transfectants derived from DHFR-TS diploid and heterozygous deletion lines. *a*, Ethidium bromide-stained gel. The samples are described in the legend to Fig. 2*a*. V marks the position of the *dhfr-ts* chromosome V; note that the karyotypes of the CBrev3 and wild-type lines differ because of an expansion of the minixon array present on chromosome 2 in the CBrev3 line¹⁶ in addition to the known *dhfr-ts* deletion on chromosome V (ref. 9) revealed by the flanking probe shown in *d*. *b*, Hybridization of the blot of the gel shown in *a* with the *neo* probe. The arrow marks the position of chromosome V. *c*, Hybridization with the *dhfr-ts* probe. *d*, Hybridization with the chromosome V-specific flanking probe. *e*, Growth of transfectant lines. Cells were inoculated into defined medium (FD+B medium¹⁰, containing 10% heat-inactivated fetal bovine serum) without thymidine unless otherwise indicated, and counted periodically. The lines were: parental CBrev3 line (□), a *dhfr-ts*⁻ derivative (E5-5C1) grown in the presence (Δ) or absence (○) of $10 \mu\text{g ml}^{-1}$ thymidine, or a clonal

derivative of the E5-5C1 line (E6-5C5) transfected with the construct pSNAR-RRv20A that expresses *dhfr-ts* (▽). METHODS. Methods of electrophoresis, hybridization and probes are described in the legend to Fig. 1. The construct pSNAR-pRRv20A was created by insertion of the 2 kb *dhfr-ts* HindIII fragment of pRRv20 (ref. 4) into the HindIII site of pSNAR, a modified derivative of pS45NEOB (ref. 5) which lacks the EcoRI site immediately upstream of *dhfr-ts* (H. Callahan, unpublished data).

for loss of DHFR-TS is thymidine auxotrophy, and when the *dhfr-ts*⁻ transfectants were added to a defined medium^{10,11} they were unable to grow in the absence of thymidine (Fig. 3*e*). Thymidine auxotrophy arose solely from deletion of *dhfr-ts*, as introduction of a circular DNA construct expressing the *dhfr-ts* enzyme into the *dhfr-ts*⁻ lines restored thymidine prototrophy (Fig. 3*e*).

The larger chromosomes hybridizing to *neo* in the complex transfectants contain homologous insertions of multiple copies of the transfecting DNA at *dhfr-ts* without replacement. All events were directed to the *dhfr-ts* chromosome, as every larger chromosome identified by the *neo* probe was also identified by both the *dhfr-ts* and flanking probes (Fig. 1, and data not shown) and probes specific for other chromosomes showed no changes. Restriction analysis of the *neo* DNA revealed the diagnostic replacement fragments and the presence of tandem repeats, as described in similar experiments in yeast^{6,12}. It is possible that the circular DNAs in the complex lines may have mediated integration into *dhfr-ts*. About two-thirds of the complex lines showed simultaneous integration of *neo* DNA into both *dhfr-ts* chromosomes (Fig. 1*b-d*, lanes 1, 3).

The efficiency of homologous gene replacement at *dhfr-ts* was high, comprising about 45% of the total transfectants analysed

(Table 1). The proportion of colonies in each class was dramatically dependent on the amount of transfecting DNA: high amounts of DNA gave mostly complex integrative events, whereas low amounts of DNA gave simple gene replacements at nearly 100% efficiency (13/14). This finding has not been previously reported in transfections of non-defective constructs, and if generally applicable it will allow experimental manipulation of the frequency of gene replacement versus integration.

Overall, the features of linear DNA transfection in *Leishmania* are similar to those reported for *S. cerevisiae*^{2,13}. Biochemical studies of *dhfr-ts*⁻ lines will facilitate analysis of the novel features of folate metabolism and antifolate sensitivity of *Leishmania*^{10,14}. Application of this powerful method to other loci, however, will require additional steps, as heterozygous deletions are usually not available. Although *Leishmania* is thought to be generally diploid^{9,11,15,16}, no sexual cycle has been demonstrated. Thus, other methods such as mitotic nondisjunction or efficient targeting with a second marker will be required. It may be possible to capitalize on a unique feature of *Leishmania* linear transfections: as simultaneous integration at both alleles is frequently observed, procedural advances may permit simultaneous replacement in a single step. □

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1. Capecchi, M. *Science* **244**, 1288-1292 (1989).
2. Rothstein, R. J. *Meth. Enzym.* **101**, 202-211 (1983).
3. Smithies, O. *et al. Nature* **317**, 230-234 (1985).
4. Kapler, G. M., Coburn, C. M. & Beverley, S. M. *Molec. cell. Biol.* **10**, 1084-1094 (1990).
5. Lebowitz, J., Coburn, C. M., McMahon-Pratt, D. & Beverley, S. M. *Proc. natn. Acad. Sci. U.S.A.*, in the press.
6. Orr-Weaver, T. L., Szostak, J. W. & Rothstein, R. J. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6354-6358 (1981).
7. Laban, A., Tobin, J. T., de Lafaille, M. A. C. & Wirth, D. F. *Nature* **343**, 572-574 (1990).
8. Beverley, S. M. *Nucleic Acids Res.* **16**, 925-938 (1988).
9. Beverley, S. M. *et al. in The Biology of Parasitism* (eds Englund P. T. & Sher A.) 431-448 (Liss, New York, 1988).
10. Petrillo-Peixoto, M. P. & Beverley, S. M. *Antimicrob. Ag. Chemother.* **31**, 1575-1578 (1978).
11. Iovannisci, D. M. & Ullman, B. J. *Parasit.* **69**, 633-636 (1983).

12. Szostak, J. W. & Wu, R. *Plasmid* **2**, 536-554 (1979).
13. Orr-Weaver, T. L., Szostak, J. W. & Rothstein, R. J. *Meth. Enzym.* **101**, 228-245 (1983).
14. Kaur, K., Coons, T., Emmett, K. & Ullman, B. J. *biol. Chem.* **263**, 7020-7028 (1988).
15. Leon, W., Fouts, F. L. & Manning, J. *Nucleic Acids Res.* **5**, 491-503 (1978).
16. Iovannisci, D. M. & Beverley, S. M. *Molec. biochem. Parasit.* **34**, 177-188 (1989).
17. Beverley, S. M., Ellenberger, T. E. & Cordingley, J. S. *Proc. natn. Acad. Sci. U.S.A.* **83**, 2584-2588 (1986).
18. Beverley, S. M., Coderre, J. A., Santi, D. V. & Schimke, R. T. *Cell* **38**, 431-439 (1984).
19. Chu, G., Vollrath, D. & Davis, R. W. *Science* **234**, 1582-1585 (1986).
20. Ellenberger, T. E. & Beverley, S. M. *J. biol. Chem.* **264**, 15094-15103 (1989).

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Targeted insertion of the neomycin phosphotransferase gene into the tubulin gene cluster of *Trypanosoma brucei*

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KINETOPLASTIDS are unicellular eukaryotes that include important parasites of man, such as trypanosomes and leishmanias. The study of these organisms received a recent boost from the development of transient transformation¹⁻⁵ allowing the short-term expression of genes reintroduced into parasites like *Trypanosoma brucei*³⁻⁵, the causative agent of African trypanosomiasis. We have obtained long-term stable transformants of *T. brucei* that have acquired the ability to grow in medium containing the drug G418, following the targeted insertion of the bacterial gene for neomycin phosphotransferase (*neo^r* gene) into the trypanosome tubulin cluster. Plasmids in which part of the *T. brucei* tubulin gene cluster has been replaced by the *neo^r* gene were used. Targeting efficiency was higher with a linearized than with a circular construct, and with 5 kilobases of tubulin gene cluster than with 0.9 kilobases. With these *neo^r* constructs homologous recombination seems to be the preferred route for insertion of exogenous DNA into the trypanosome genome, allowing gene targeting without counter-selection.

Most of the tubulin genes of *T. brucei* are arranged in two allelic clusters of alternating α and β tubulin genes^{6,7} (Fig. 1a). We constructed two gene-targeting plasmids, pUCTbneo2 and pUCTbneo3, containing an in-frame fusion of the *T. brucei* β tubulin gene and the *Escherichia coli neo^r* gene (Fig. 1b). In these constructs, the *neo^r* gene is flanked by sequences required for proper expression and allowing its insertion into the genomic tubulin cluster by homologous recombination.

The pUCTbneo2 and pUCTbneo3 plasmids were introduced into procyclic trypanosomes by electroporation⁵, either as supercoiled DNA or after linearization by digestion in the vector. In all instances, trypanosomes growing in the presence of G418 (50 $\mu\text{g ml}^{-1}$) were observed after 6–12 days. Wild-type trypanosomes are completely killed after 5–6 days by this concentration of G418 and spontaneous resistance of *T. brucei* to G418 has not been observed. Stable transformants were obtained with linearized pUCTbneo3 at a frequency of ~ 1 in 10^5 trypanosomes, as determined by limiting dilution. As $\sim 5 \times 10^6$ trypanosomes were used per transformation, the transformed populations should initially be heterogeneous. This was indeed observed (data not shown). Most populations became clonal, however, after prolonged diluted passaging under G418 selection.

Several diagnostic restriction digests (Fig. 1a) were used to test whether the *neo^r* gene had been inserted into the tubulin gene cluster. The pattern characteristic of a single insertion of the *neo^r* gene in the tubulin gene cluster, was found for transformants pUCTbneo2-circular (Fig. 2, lanes 2) and pUCTbneo3-linear (Fig. 2, lanes 4). The diagnostic bands hybridizing in the *NotI* digests (Fig. 2a and b) were also obtained with restriction endonucleases *EcoRV* and *BamHI* (data not shown). The *NcoI* digests (Fig. 2c and d) show that the insertion of the *neo^r* gene in the pUCTbneo2-circular transformant (Fig. 2, lanes 2) must have been close to the edge of a cluster, as one fragment (Y in Fig. 1a) is large and in the compression zone and the other (X in Fig. 1a) only 7.4 kilobases (kb). The fact that transformant

pUCTbneo3-linear yields four large *NcoI* fragments (Fig. 2c and d, lanes 4), instead of the two border fragments predicted, indicates that this transformant is not (yet) clonal, but contains trypanosomes with the *neo^r* gene inserted in different positions in the tubulin gene cluster. Transformant pUCTbneo2-linear (Fig. 2, lanes 1) gave the pattern characteristic of replacement of two neighbouring β tubulin genes by *neo^r*. The *NcoI* digest indicates again that the *neo^r* gene was inserted close to the edge of the tubulin gene cluster.

Although the results in Fig. 2 show that the *neo^r* gene is present in a tubulin gene cluster, they do not prove that this is one of the normal chromosomal arrays rather than an excised or displaced copy. To exclude this latter possibility, the *NcoI* digests were size-fractionated by transverse alternating field electrophoresis to resolve the *NcoI* fragments containing the wild-type tubulin gene clusters. These clusters are present in DNA fragments of 40 and 70 kb (Fig. 3, lane 3) and integration

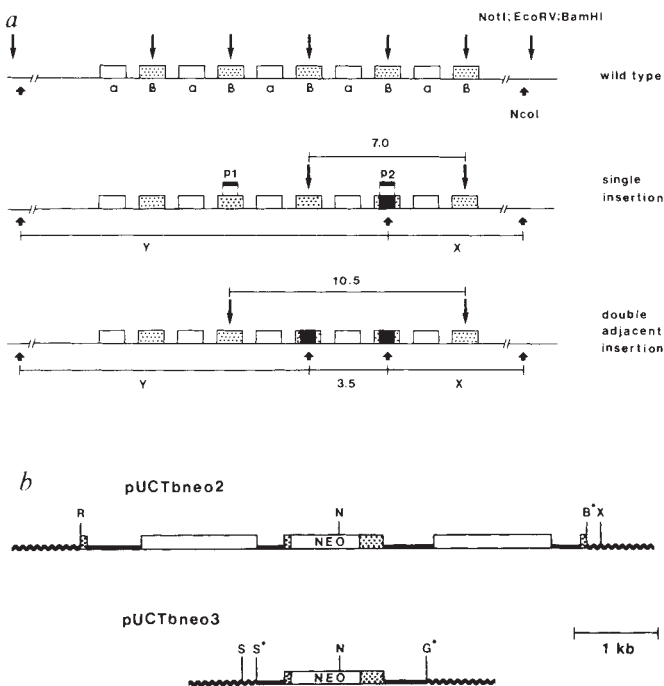
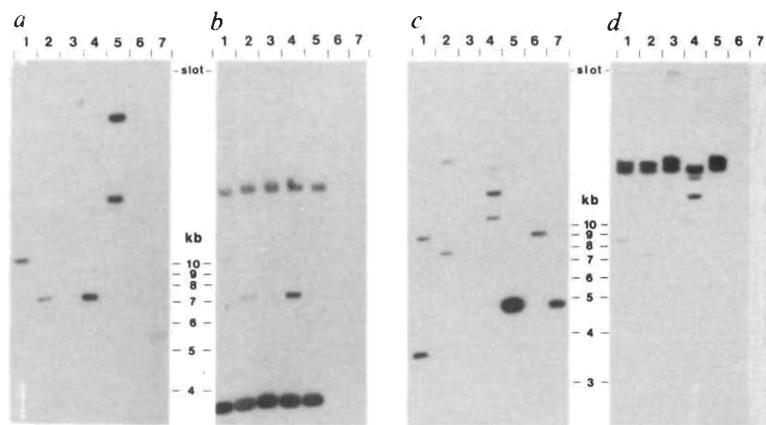


FIG. 1 Plasmid constructs pUCTbneo2 and pUCTbneo3 and diagnostic restriction enzyme digests for homologous integration of the *neo^r* gene into the tubulin gene cluster. **a**, Restriction endonucleases *NotI*, *EcoRV* and *BamHI* (indicated by the large arrows above line) cut the β tubulin gene in the segment replaced by the *neo^r* gene. This replacement leads to the generation of a new fragment of 7.0 kb (single insertion of the *neo^r* gene) or 10.5 kb (double adjacent insertion), hybridizing to the β tubulin-specific probe (p1) as well as the *neo^r* gene probe (p2). *NcoI* (small arrows below line) does not cut the tubulin clusters, but it has a single recognition site in the *neo^r* gene. Insertion of a single *neo^r* gene results in two border fragments hybridizing to both probes; replacement of two neighbouring β tubulin genes generates, in addition, a 3.5 kb fragment that only hybridizes to the *neo^r*-specific probe. **b**, Plasmids pUCTbneo2 and pUCTbneo3 were derived by stepwise cloning. The 6.14-kb *EcoRI/BclI* fragment of pTb $\alpha\beta$ T-1 (ref. 7) was cloned, via *EcoRI/HindIII* and *HindIII/BclI* fragments, into the *EcoRI* and *BamHI* restriction sites of pUC18. Subsequently, the *BamHI/SmaI* fragment of pKm109.90 (ref. 11) containing the *neo^r* gene, was cloned into the *BclI* and *EcoRV* restriction sites of the β tubulin gene, giving rise to plasmid pUCTbneo2. Cloning of the *Scal/BglII* fragment of pUCTbneo2 into the *SmaI* and *BamHI* restriction sites of pUC18 yielded plasmid pUCTbneo3. Plasmid pUCTbneo2 was linearized with *XbaI*, pUCTbneo3 with *SacI*. Transfections were performed with 50 μg circular DNA, or 8 μg linear DNA, exactly as described⁵; 48 h after transfection, G418 was added to a final concentration of 50 $\mu\text{g ml}^{-1}$. Dotted boxes, β -tubulin sequences; NEO, *neo^r* gene; open boxes, α tubulin genes; thick lines, tubulin intergenic regions; wavy lines, vector sequences. Abbreviations: B*, *BamHI/BclI* fusion; G*, *BglII/BamHI* fusion; S*, *Scal/SmaI* fusion; N, *NcoI*; R, *EcoRI*; S, *SacI*; X, *XbaI*.

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FIG. 2 DNA blot analysis of G418-resistant mutants transfected with the *neo^r*-tubulin constructs. Nuclear DNA was isolated¹² from G418-resistant trypanosomes transfected with pUCTbneo2 or 3 and digested with restriction enzymes *Not*I (a and b) or *Nco*I (c and d). The digests were size-fractionated in a 0.5% agarose gel, transferred to nitrocellulose filters and analysed by hybridization to the *Bam*HI/*Sma*I *neo^r*-specific probe (probe p2 in Fig. 1a) in a and c and subsequently, after stripping off this probe, by hybridization to the *Bcl*I/*Eco*RV β tubulin probe (probe p1 in Fig. 1a) in b and d. Probe labelling, hybridization and autoradiography were as described⁵. Blots were washed at a stringency of $0.1 \times \text{SSC}$, 0.1% SDS at 65°C. The following DNAs were used: lane 1, transfectant with linear pUCTbneo2; lane 2, transfectant with circular pUCTbneo2; lane 3, non-transfected trypanosomes; lane 4, transfectant with linear pUCTbneo3; lane 5, transfectant with circular pUCTbneo3; lane 6, plasmid pUCTbneo2; lane 7, plasmid pUCTbneo3.



of a *neo^r* gene results in a reduction of the size of the larger fragment in each of the transformants. The pUCTbneo2-circular (Fig. 3, lane 2) and the pUCTbneo2-linear (Fig. 3, lane 1) transformants seem to be clonal, as the *Nco*I fragments add up to the 70-kb wild-type fragment. In DNA of the pUCTbneo3-linear transformant (Fig. 3, lane 4) the four large fragments shown in Fig. 2 seem to be the result of *Nco*I digestion of only the large tubulin gene cluster. This indicates that this transformant is a mixed population of two different homologous recombinants, both in the large tubulin gene cluster and towards the centre of the cluster, albeit in different positions.

With linear constructs, a single homologous recombination event would result in a chromosome break. As we have not observed altered migration of either of the chromosome-sized DNA molecules containing the tubulin gene arrays in pulsed field gel electrophoresis (data not shown), such breaks probably do not occur. Insertion of the *neo^r* gene must therefore either occur by a double homologous recombination or by a gene conversion event. The size of the homologous segment in the incoming DNA plays a part in the efficiency of insertion, as we have obtained insertion of circular DNA with 5 kb of tubulin

gene sequences, but not with 0.9 kb, although 0.9 kb was sufficient after linearization of the plasmid.

The single *neo^r* gene insert in the tubulin cluster of transformant pUCTbneo2-circular was found to be stable during growth in the absence of G418 for 5 months (300 doublings). To test whether this gene could be amplified under increased drug selection, the trypanosomes were stepwise adapted to growth in 100, 200 and 500 μg G418 ml^{-1} , respectively. Extra *neo^r* gene copies were already noticeable by 100 μg G418 ml^{-1} and these were also in standard tubulin arrays, rather than in extrachromosomal copies (data not shown).

The circular form of the pUCTbneo3 plasmid was not found to insert into the genome. Instead, the transformant contained an extrachromosomal circular oligomer of the transforming plasmid, which was lost on removal of the drug selection (data not shown). Therefore, a plasmid containing less than 1 kb of tubulin gene sequence contains all the information required for extrachromosomal replication and the proper expression of the *neo^r* gene. A similar result had been obtained in the kinetoplastids *Leishmania enrietti*⁸ and *Leptomonas seymouri* (V. Bellofatto, personal communication) with constructs containing the *neo^r* gene and the α tubulin intergenic region.

In our experiments with *neo^r*-tubulin constructs we have not found a clear example of non-homologous insertion of the *neo^r* gene into the trypanosome genome. Homologous recombination therefore seems to be the preferred route for insertion of genes into the trypanosome genome. As this has also been observed for yeast⁹ and other fungi¹⁰, it may be a general feature of the simple genome of unicellular eukaryotes.

The ability to introduce new genes stably and disrupt endogenous ones completes the arsenal of reverse genetic techniques required for efficient study of gene expression in trypanosomes. □

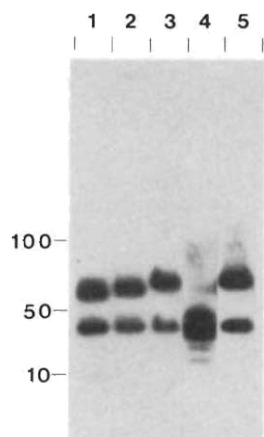


FIG. 3 DNA blot analysis of the location of the *neo^r* gene within the tubulin gene clusters. Samples of the G418-resistant transfected trypanosomes were prepared as described¹³ and digested with the restriction enzyme *Nco*I. DNA fragments generated were resolved with transverse alternating field electrophoresis in a 1% agarose gel in a buffer containing 10 mM Tris, 0.5 mM EDTA, pH 7.5, over a 24 h period at 14°C; the electric field (200 V, 160 mA) was switched every 4 s. The blot was analysed by hybridization to the β tubulin-specific probe as in Fig. 2. The following DNAs were used: lane 1, transfectant with linear pUCTbneo2; lane 2, transfectant with circular pUCTbneo2; lane 3, non-transfected trypanosomes; lane 4, transfectant with linear pUCTbneo3; lane 5, transfectant with circular pUCTbneo3. Size markers (kb) on the left.

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1. Bellofatto, V. & Cross, G. A. M. *Science* **244**, 1167-1169 (1989).
2. Laban, A. & Wirth, D. F. *Proc. natn. Acad. Sci. U.S.A.* **86**, 9119-9123 (1989).
3. Rudenko, G. et al. *Molec. Cell. Biol.* **10**, 3492-3504 (1990).
4. Clayton, C. E. et al. *Molec. Cell. Biol.* **10**, 3036-3047 (1990).
5. Zomerijk, J. C. B. M. et al. *EMBO J.* **9**, 2791-2801 (1990).
6. Seebeck, T., Whittaker, P. A., Imboden, M. A., Hardman, N. & Braun, R. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4634-4638 (1983).
7. Thomashow, L. S., Milhausen, M., Rutter, W. J. & Agabian, N. *Cell* **32**, 35-43 (1983).
8. Laban, A., Tobin, J. F., Curotto de Lafaille, M. A. & Wirth, D. F. *Nature* **343**, 572-574 (1990).
9. Hinnen, A., Hicks, J. B. & Fink, G. R. et al. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1929-1933 (1978).
10. Timberlake, W. E. & Marshall, M. A. *Science* **244**, 1313-1317 (1989).
11. Reiss, B., Sprengel, R. & Schaller, H. *EMBO J.* **3**, 3317-3322 (1984).
12. Bernards, A. et al. *Cell* **27**, 497-505 (1981).
13. Van der Ploeg, L. H. T., Schwartz, D. C., Cantor, C. R. & Borst, P. *Cell* **37**, 77-84 (1984).

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An unusually large multifunctional polypeptide in the erythromycin-producing polyketide synthase of *Saccharopolyspora erythraea*

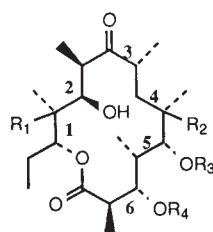
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ERYTHROMYCIN A, a clinically important polyketide antibiotic, is produced by the Gram-positive bacterium *Saccharopolyspora erythraea*. In an arrangement that seems to be generally true of antibiotic biosynthetic genes in *Streptomyces* and related bacteria like *S. erythraea*¹, the *ery* genes encoding the biosynthetic pathway to erythromycin are clustered around the gene (*ermE*) that confers self-resistance on *S. erythraea*²⁻⁶. The aglycone core of erythromycin A is derived from one propionyl-CoA and six methylmalonyl-CoA units, which are incorporated head-to-tail⁷⁻¹⁰ into the growing polyketide chain, in a process similar to that of fatty-acid biosynthesis¹, to generate a macrolide intermediate, 6-deoxyerythronolide B¹⁰. 6-Deoxyerythronolide B is converted into erythromycin A through the action^{2-5,10} of specific hydroxylases, glycosyltransferases and a methyltransferase. We report here the analysis of about 10 kilobases of DNA from *S. erythraea*, cloned by chromosome 'walking' outwards from the erythromycin-resistance determinant *ermE*, and previously shown to be essential for erythromycin biosynthesis^{5,11}. Partial sequencing of this region¹² indicates that it encodes the synthase. Our results confirm this, and reveal a novel organization of the erythromycin-producing polyketide synthase, which provides further insight into the mechanism of chain assembly.

The structures of 6-deoxyerythronolide B (I) and the aglycone core of erythromycin A (II) are shown in Fig. 1. An unusual feature of the part of the *ery* gene cluster shown in Fig. 2 is the presence of an open reading frame (ORF A), extending over 9.5 kilobases (kb) of DNA. The predicted gene product would contain 3,178 amino acids, and would have a relative molecular mass (M_r) of 332,472. Comparison with available protein sequence databases reveals that nine separate portions of the predicted amino-acid sequence (Fig. 2) are very similar to active-site sequences for the constituent catalytic activities of known fatty acid synthases and polyketide synthases¹³⁻¹⁹. Alignments between parts of the ORF A gene product and active site sequences for acyl carrier proteins (ACPs), β -ketoacyl-ACP synthases,



I 6-deoxyerythronolide B ($R_1=R_2=R_3=R_4=H$)

II erythromycin A ($R_1=R_2=OH, R_3=desosamine, R_4=cladinose$)

FIG. 1 Structures of 6-deoxyerythronolide B (I) and erythromycin A (II). The new carbon-carbon bonds made in each successive chain extension cycle are numbered 1-6.

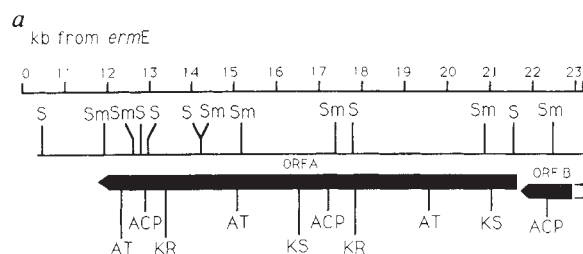


FIG. 2 a, Restriction map of part of the erythromycin biosynthetic gene cluster, encoding components of the polyketide synthase. The direction of transcription of the open reading frames deduced from analysis of the nucleotide sequence is indicated. S, *SacI*; Sm, *SmaI*. Relative positions of potential catalytic activities are indicated by: ACP, acyl carrier protein; KS, β -ketoacyl-ACP synthase; AT, acyltransferase or thioesterase; KR, β -ketoacyl-ACP reductase. b, (opposite) Predicted amino-acid sequence (single-letter code) for *ery* ORF A. Potential active-site sequences are underlined. Numbers, positions of the amino-acid residues. The complete nucleotide sequence relevant to the present paper has been submitted to the EMBL database under accession number X56107.

METHODS. A library of *S. erythraea* size-fractionated genomic DNA in λ EMBL3 (ref. 25) was screened using a nick-translated *EcoRI* fragment containing the *ermE* resistance gene. A clone (insert size 20 kb) extending about 25 kb downstream of *ermE* was used for sequence analysis. All sequence was obtained by double-stranded plasmid sequencing^{6,24} using Sequenase (USB).

acyltransferases (including thioesterases) and β -ketoacyl-ACP reductases are shown in Fig. 3b. Another ORF, ORF B, the full length and sequence of which are not yet known, lies immediately upstream of ORF A. The C-terminal portion of the predicted product of ORF B also contains a sequence similar to that of known ACP active-site sequences (Fig. 2a).

Several important points emerge from this analysis. The marked similarity between the product of ORF A and known fatty acid and polyketide synthases strongly reinforces the earlier conclusions from genetic studies^{5,11} and from partial sequencing¹² that this DNA encodes the polyketide synthase. The N- and C-terminal halves of the ORF A gene product each contain an identical set of four deduced activities in the same order along the polypeptide chain, except for an additional C-terminal domain predicted to have acyltransferase or thioesterase activity (Fig. 2). The observed order of these four catalytic activities is precisely that found in vertebrate fatty acid synthases^{18,19}. The similarity to vertebrate fatty acid synthases extends much further than the identifiable active-site sequences, as illustrated by a DIAGON²⁰ plot (Fig. 3a).

Also summarized in Fig. 3b are the evident similarities between the predicted ORF A gene product and active-site sequences that have been found in recently sequenced polyketide synthase genes from *Streptomyces* spp. that produce tetracenomycin¹⁴ and granaticin¹⁵, both of which are aromatic polyketides constructed exclusively from acetate units. These clusters contain single copies of genes that code for apparently monofunctional proteins, an arrangement analogous to the dissociable (type II) fatty acid synthase found in *Escherichia coli*¹³ and in most other bacteria¹. These findings clearly demonstrated the long-suspected link between bacterial polyketide and fatty acid synthases, but, as our results show, bacterial polyketide synthases are not necessarily complexes of monofunctional polypeptides.

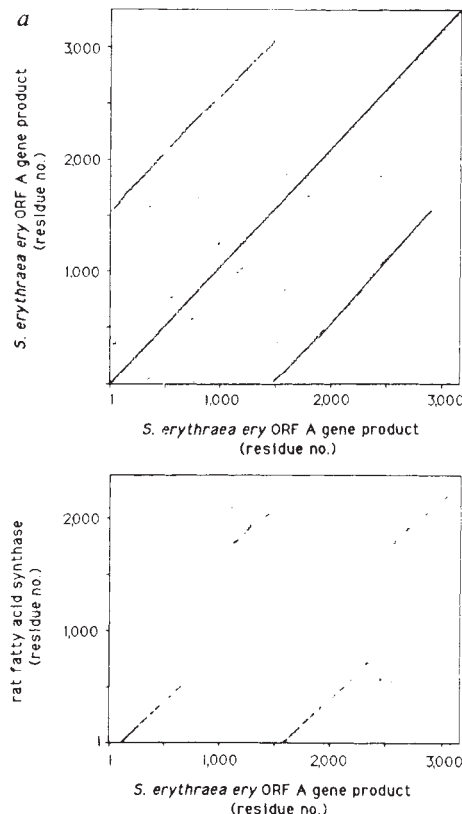
The processive^{9,10} assembly of the polyketide chain of 6-deoxyerythronolide B requires six consecutive cycles of condensation and reduction (Fig. 1). Each half of the ORF A gene product seems to possess the appropriate activities to extend and then reduce the polyketide chain to a β -hydroxyacyl-thioester, the redox level found after extension cycles 1, 2, 5 and 6 (see Fig. 1 legend). The reduction step would involve the ketoreductase labelled KR in Fig. 2a. The presence of the C-

b 1 MSGDNGMTEE KLRRLKRTV TELDSVTARL REVEHRAGEP IATVGMACRF
 51 PGDVSDEPST WEFVSGGDA IAEAPADRGW EPDPDARLGG MIAAAGDFDA
 101 GFFGISPREA LAMPDQQRIM LEISWEALER AGHDPVSLRG SATGVFTCVG
 151 TVDYGPRPDE APDEVLYGVG TGTAASSVAG RVAYCLGLEG PAMTVDTACS
 201 SGLTALHIAE ESLRRDECGL ALAGGVTVMS SPGAFTEFRS OGGLAADGRC
 251 KPFSKAADGF CLAEAGVLV LQLLSAARRE GRPVLAVLAG SAVNQDGSN
 301 GLTAPSGPAQ QRVIRRALEN AGVRAGVDYD VEAHGTGTRL GDPFIEVHALL
 351 STYGAERDPE DPLWIGSVKS NIGHTQAAAG VAGVMKAVLA LRHGEMPRTL
 401 HFDEPSQIE WDLGAVSVVS QARSWPAGER PRAGVSSFG ISGTHAHVIV
 451 EEAPEADEFE PAPDSGPVPL VLSSGRDEQAM RAQAGRLADH LAASRGTRCA
 501 TPVSRWPPAA APWEQVVVDG RDEALAGLRA VADRRIADRT ATGQGNPSPR
 551 RVAMVFPGQG AQWQGMARDL LRESQVFADS IRDCERLAP HVDWLSLTDLL
 601 SGARPLDRVD VVQALFAVM VSLAALWRSH GVEPAAYVGH SOGEYAAAHV
 651 AGAITLEDAA KLVAVSRVL RRLGGQGGMA SFLGTEQAA ERIGRFAGAL
 701 SIASVNGPRS VVVVAGESGP LDELIAECEA EAHKARRIPV DYASHSPQVE
 751 SLRELLTEL AGISPVADV ALYSTTTGQP IDTATMDTAY WYANLREQVR
 801 FQDATRLAE AGFDAPFEVS PHPVLTGVIE ATLDLSALPAD AGACVVGTLR
 851 RDRGGLADFH TALGEAYAQG VEVDWSFAFA DARPVELPVY PFQRELFPVY
 901 FQRQRYWLP I PTGGRARDED DDWRYOVVWR EAEWESASLA GRVLLVTGPG
 951 VPSELSDAIR SGLEQSGATV LTCDVESRST IGTALEAADT DALSTVGVAA
 1001 VPHGEAVDPS LDALALVQAL GAAGVEAPLV VLTNRNAVQA DGLVDPQAQ
 1051 MVGGGLRVVG IEQPGRWGGL VDLVDADAAS IRSLAVALAD PRGEEQVAIR
 1101 ADGKIKVARLV PAPAARATHP LEPLAGTVLV TGGIGAHLAR WLAASGAEHL
 1151 VLLGRRGADA PGASELREEL TALGTGVITA ACQVADRRL EAVLAEEAAA
 1201 EGRTVSAMVM AAGVSTSTPL DDLTEAEFTE IADVKVRGTV NDELCPDLD
 1251 AFVLFSSNAC VHGSPGLASY AAANAFIDGF AARASEGAP VTSIAWLWA
 1301 GQNMAGDEGG EYLRSGQLRA MDPRAVEEL HITLDHGQTS VSVVMDRRR
 1351 FVELFTAAHR RPLFDEIAGA RAEARQSEEG PALAQLRAL LCGDREHRL
 1401 AHLIRAEVAA VLGHGDDAAI DRDRAFRDLG FDSMTAVDLR NRIAAVTGVR
 1451 EAATVVDHP TITRIADHYL ERLVGAAEAE QAPALVREVP PKDADDPIAI
 1501 VGMACRFPGG VHNPGELWEF IVGGGDVATE MPTDRGWLD ALFDPDPQRH
 1551 GTSYSRHGAF LDGAADFDA FFGISPREAL AMPDQQRQVL ETTWELFENA
 1601 GIDPHSVRGS DTGVFLGAAY QGYQGDVAVP EDSEGYLLTG NSSAVVSGRV
 1651 AYVLGLGEPV VTDVTCSSS LVALLHAGCS LRDGDCGLAV AGGVSVMAGP
 1701 EVFTEFSRQG GLAVDGRCKA FSAEADGFLG PEGVAVVQIQ RLSGDAEAG
 1751 RQVLGVVAGS AINQDQATNG LAAPSGVAAQ RVIRKAWARA GITGADVAVV
 1801 EAHGTGTRLG DPVEASALLA TYGKSRGSSG PVLLGSVXSN IGHAQAAAGV
 1851 AGVJKVVLGL NRGVLPPMLC RGRSPLIEW SSGGVLEAEA VSPWPPAADG
 1901 VRRAGVSAGF VSGTNAHVIT AEPPEPEPLP EPGPVGVLA ANSVPVLLSA
 1951 RTETALAAQA RLLESAVDDS VPLTALASAL ATGRAHLPRR AALLAGDHEQ
 2001 LRGLRAVAE GVAAPGATG TASAGGVVVF FPGQGAQWEG MARGLLSVPV
 2051 FAESIAECOA VLSEVAGFSA SEVLEQRPDA FSLERVDVQ PVLFVSMVSL
 2101 ARLWAGCGVS PSAYIGHSSQ ELAAAVVAGV LSLDGVVVV ALRAKALRAL
 2151 AGKGMVSLA PAEPAPGDGE AFVACLGADG HEDQVDIRDH ARYGRVLVRA
 2201 VARGEDGVR AKTLFVDYAS HSRHVEEIRL TILADLDGIS ARRAAIPLYS
 2251 TLHGERRDMG PRYWDNLRS QVRFEAVSA QSPDGHATFV EMSPHPVLA
 2301 AVQEIADAV AIGSLHRDTA EEHLIAELAR AHVHGVAVDW RNVFPAAPV
 2351 ALPNYPFEPQ RYWLAEVSD QLADSRVYRD WRPLATTPVD LEGGFLVHGS
 2401 APESLTSAVE KAGGVFVAS ADREALAAL REVPGEVAGV LSVHTGAANA
 2451 LALHQSLEGA GVRAPLWLV SRAVALGESE PVDPEQAMV GLGRVYMGLET
 2501 PERWGLVDL PAEPAPGDGE AFVACLGADG HEDQVDIRDH ARYGRVLVRA
 2551 PLGTRESSWE PAGTALVTGG TGALGGHVAR HLCRCGVEDL VLVSRRGVDA
 2601 PAAAELEAL VALGPKTTIT ACQVADRQL SKLLEELRGQ GRPVRTVVHT
 2651 AGVPESRPLH ETGELESVCA AKVTGARLLD ELCPDAETEV LESSAGGVWG
 2701 SANLGAYSAA NAYLDALAHN AGRKAVRRTS VAWGAWAGEG MATGDELEGT
 2751 RRLGRPMAPD RAI RALHQAAL DNGDTCVSTA DWDKEAFVAG FTAARPRPL
 2801 DELVTFPAGA VPAPQAAPOL EMTSQELLEF THSHVAAILG HSSPDVAGQD
 2851 QPFTLGEDS LTAVGLNOL QOATGLALPA TLVFEHPTVR RLADHIGQOL
 2901 DSGTPAREAS SALRDGYRQA GVSGRVRSYL DLLAGLSDFR EHFDSGQFS
 2951 LDLDVMDAGP GEVTVICCAQ TAAISGPHEF TRLAGALRGI APVRAVPQPG
 3001 YEEGEPPLSS MAAVAQVAD AVIRTQDQKE FVYVAGHSAGA LMAYALATEL
 3051 LDRGHPPRGV VLIQVPPGH QDAMNALLEE LTATLFDRET VRMDTDLTA
 3101 LGAYDIRLTQ WRPRETGLPT LLVSAGEPMG PWPDDSWKPT WPFEDHTVAV
 3151 PGDFTMVQE HADAIAHRLD AWLGGGNS*

FIG. 2b

terminal 'thioesterase' domain suggests that this additional activity might be required for lactonization of the completed chain, in which case the polypeptide encoded by ORF A would probably catalyse cycles 5 and 6 in the assembly of the chain from propionate units.

The ORF A gene product might catalyse more than two of the six cycles, and further sequence analysis of DNA adjacent to ORF A is required to establish the overall organization of the synthase. Convincing evidence has been obtained for a head-to-tail arrangement of the two identical multifunctional subunits in vertebrate fatty acid synthases²¹. If the two halves of the ORF A gene product folded back on each other, it would bring both the predicted β -ketoacyl-ACP synthase active sites into close proximity to an ACP domain in an analogous way. No information is yet available for other reduced bacterial polyketides, but this novel arrangement of a multifunctional polypeptide provides an attractive model for such systems. There are no real clues yet to the means by which a growing acyl chain might be transferred between active sites, or between the ORF A gene product and other components of the polyketide synthase. In any event, our new-found knowledge of the structure of the 6-deoxyerythronolide synthase should be a helpful guide in the detection and eventual purification of this enzyme.



b

(i)

ORF A (1246)

ORF A (2683)

chick

rat

C	P	D	L	D	A	F	V	L	F	S	S	N	A	G	V	W	G
C	P	D	A	E	T	F	V	L	F	S	S	G	A	G	V	W	G
C	P	D	L	D	Y	F	V	V	F	S	S	V	S	C	G	R	G
C	P	E	L	D	Y	F	V	A	F	S	S	V	S	C	G	R	G

(ii)

ORF A (190)

ORF A (1658)

chick

tcmI

gra

G	P	A	M	T	V	D	T	A	C	S	S	S	G	L	T
G	P	A	V	T	V	D	T	A	C	S	S	S	S	L	V
G	P	S	L	T	I	D	T	A	C	S	S	S	S	L	M
G	P	V	T	V	S	T	G	C	T	S	G	L	D		
G	P	V	T	M	V	S	D	G	C	T	S	G	L	D	

(iii)

ORF A (1428)

ORF A (2855)

chick

tcmI

gra

E. coli

D	L	G	F	D	S	M	T	A	V	D	L	R	N	R
E	L	G	F	D	S	L	T	A	V	G	L	R	N	Q
D	L	G	F	D	S	L	A	A	V	E	L	R	N	R
D	L	G	L	D	S	L	M	G	V	E	V	R	Q	T
D	L	G	Y	D	S	I	A	L	E	V	E	I	S	A
E	L	G	Y	D	S	L	A	L	M	E	S	A	S	R
D	L	G	A	D	S	L	D	T	V	E	L	V	M	A

(iv)

ORF A (634)

ORF A (2111)

ORF A (3030)

chick ac/mal

P	A	A	V	V	G	H	S	Q	G	E	I	A	A	A
P	S	A	V	I	G	H	S	Q	G	E	I	A	A	A
F	F	V	V	A	G	H	S	A	G	A	L	M	A	Y
G	I	L	G	H	S	Q	G	L	V	T	A	V		

FIG. 3 Comparison of *S. erythraea* ORF A gene product with known fatty acid and polyketide synthases. a, Comparison of the amino-acid sequence of *S. erythraea* ery ORF A gene product with itself and with the sequence of rat fatty acid synthase¹⁷, using DIAGON²⁰ plots. The comparison used a 41-residue segment and generated a dot if the score exceeded 455 (ref. 20). b, Alignment of portions of the amino-acid sequence of ORF A and ORF B gene products with active-site sequences from (i) β -ketoacyl-ACP reductases, (ii) β -ketoacyl ACP synthases (iii) acyl carrier proteins (ACP) and (iv) acyltransferases/thioesterases. ORF A, *S. erythraea* ery ORF A gene product; ORF B, *S. erythraea* ery ORF B gene product; tcmI, *Streptomyces glaucescens* tetracenomycin ORF I (ref. 14); gra, *Streptomyces violaceoruber* dihydrogranaticin ORF I gene product (ref. 15); chick ac/mal, acetyl/malonyltransferase from chicken fatty acid synthase¹⁸; rat, rat fatty acid synthase¹⁷. The position of each sequence in the ORF A gene product is shown in brackets.

METHODS. The SWISSPROT protein database was searched using the programme FASTA²⁶ and the EMBL and NEWEMBL nucleic acid databases were searched using the programme TFasta²⁶.

The remarkable size of ORFA places the 6-deoxyerythronolide synthase of *S. erythraea* in a select group of enzymes that synthesize secondary metabolites using giant, multifunctional polypeptides. The non-ribosomal peptide synthases produced by fungi and bacteria are well represented in this group^{22,23} and provide the most spectacular example, the single polypeptide chain of cyclosporin synthase from the fungus *Beauveria nivea*, which has a reported M_r of ~800,000 (ref. 22). Peptide synthases^{22,23} and polyketide synthases¹ both adopt a strategy in which the growing chain is linked to a potentially mobile 4'-phosphopantetheinyl attachment site on the protein. The structural basis for this interesting mechanistic similarity between the two groups of enzymes can now be explored more fully. □

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1. Sherman, D. H. & Hopwood, D. A. *Rev. Genet.* **24**, (in the press).
2. Stanzak, R., Matsushima, P., Baltz, R. H. & Rao, R. N. B. *Bio/technology* **4**, 229-232 (1986).
3. Weber, J. M. & Losick, R. *Gene* **68**, 173-180 (1988).
4. Weber, J. M., Wierman, C. K. & Hutchinson, C. R. *J. Bact.* **164**, 425-433 (1985).
5. Weber, J. M., Leung, J. O., Maine, G. T., Potenz, R. H. B., Paulus, T. J. & DeWitt, J. P. *J. Bact.* **172**, 2372-2383 (1990).

6. Dhillon, N., Hale, R. S., Cortes, J. & Leadlay, P. F. *Molec. Microbiol.* **3**, 1405-1414 (1989).
7. Kaneda, T., Butte, J. C., Taubman, S. B. & Corcoran, J. W. *J. Biol. Chem.* **237**, 322-328 (1962).
8. Cane, D. E., Hasler, H. & Liang, T.-C. *J. Am. Chem. Soc.* **103**, 5960-5962 (1981).
9. Cane, D. E., Liang, T.-C., Taylor, P. B., Chang, C. & Yang, C.-C. *J. Am. Chem. Soc.* **108**, 4957-4964 (1986).
10. Seno, E. T. & Hutchinson, C. R. in *The Bacteria Vol IX* (eds Queener, S. W. & Day, L. E) 231-279 (Academic, New York, 1986).
11. Tuan, J. S. *et al. Gene* **90**, 21-29 (1990).
12. Donadio, S. *et al.* in: *Genetics and Microbiology of Industrial Microorganisms* (eds Hershberger, C. L., Queener, S. W. & Hageman, G.) 53-59 (Am. Soc. of Microbiol., Washington, DC 1989).
13. Kauppinen, S., Siggaard-Andersen, M. & von Wettstein-Knowles, P. *Carlsberg Res. Commun.* **53**, 357-370 (1988).
14. Bibb, M. J., Biro, S., Motamedi, H., Collins, J. F. & Hutchinson, C. R. *EMBO J.* **8**, 2727-2736 (1989).
15. Sherman, D. H. *et al. EMBO J.* **8**, 2727-2735 (1989).
16. Schweizer, M. *et al. Molec. gen. genet.* **203**, 479-486 (1986).
17. Schweizer, M., Takabayashi, K., Laux, T., Beck, K. F. & Schreglmann, R. *Nucleic Acids Res.* **17**, 567-586 (1988).
18. Chirala, S. S. *et al. J. Biol. Chem.* **264**, 3750-3757 (1989).
19. Mohamed, A. H., Chirala, S. S., Mody, N. H., Huang, W.-Y. & Wakil, S. J. *J. Biol. Chem.* **263**, 12315-12325 (1988).
20. Staden, R. *Nucleic Acids Res.* **12**, 521-528 (1984).
21. Wakil, S. J. *Biochemistry* **28**, 4523-4530 (1989).
22. Lawen, A. & Zocher, R. *J. Biol. Chem.* **265**, 11355-11360 (1990).
23. Skarpeid, H.-J., Zimmer, T.-L. & Von Doehren, H. *Eur. J. Biochem.* **189**, 517-522 (1990).
24. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463-5467 (1977).
25. Frischauf, A.-M., Garoff, H. & Lehrach, H. *Nucleic Acids Res.* **8**, 5541-5549 (1980).
26. Devereux, J., Haeblerli, P. & Smithies, O. *Nucleic Acids Res.* **12**, 387-395 (1984).

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Bidirectional incompatibility between conspecific populations of *Drosophila simulans*

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CYTOPLASMIC incompatibility (CI) describes the phenomenon whereby eggs fertilized by sperm from insects infected with a rickettsial endosymbiont fail to hatch. Unidirectional CI between conspecific populations of insects is a well documented phenomenon¹⁻⁹. Bidirectional CI has, however, only been described in mosquito populations¹⁰, and recently between closely related species of parasitic wasps¹¹, where it is of interest as both an unusual form of reproductive isolation¹ and as a potential means of insect population suppression¹²⁻¹⁵. Here we report on the first known example of bidirectional CI between conspecific populations of *Drosophila simulans*. Further, we show that defects as early as the first cleavage division are associated with CI. This observation suggests that the cellular basis of CI involves disruption of processes before or during zygote formation and that CI arises from defects in the structure and/or function of the sperm during fertilization.

Strains of *D. simulans* that display unidirectional CI have previously been identified⁸. Utilizing one of these infected strains originally taken from Riverside, California and a strain collected in Hawaii, it was possible to induce intrastrain unidirectional CI within each strain by crossing tetracycline-treated females with untreated males (Table 1). The pattern of this incompatibility implies that both strains are infected with endosymbionts. Of particular significance however, is the observation that these two strains also exhibit interstrain bidirectional CI when crossed (Table 1). When these strains were treated with the antibiotic tetracycline hydrochloride, the incompatibility was removed and progeny counts returned to normal. This result suggests that microorganisms are involved in the expression of both bidirectional and unidirectional CI. For bidirectional CI to be expressed however, indicates that the endosymbionts are likely to have diverged significantly in these two populations of flies.

The presence of microorganisms was verified by direct epifluorescent observation of fixed, DAPI (4,6-diamidino-2-phenylindole)-stained eggs. Figure 1a shows large quantities of small,

punctate DNA-positive bodies in the cortex of the eggs of untreated flies. We assume these bodies represent the DNA present in the rickettsial symbiont on the basis of our observations that (1) such bodies are absent in eggs derived from mothers that had previously been treated with tetracycline (see below); (2) they are absent in other strains of *Drosophila* (our unpublished observations); and (3) they are correlated with transmission electron microscope observations of rickettsial infection in the males from the Riverside strain of *D. simulans*¹⁶. During the early cleavage stages of development, rickettsiae are observed in the regions surrounding the nuclei (Fig. 1b). This association with nuclei continues until the blastoderm stages, including incorporation of rickettsiae into pole cells as shown in Fig. 1c. Pole buds and pole cells are identified by Nomarski observation of the posterior end of the egg¹⁷ (Fig. 1c, left), and the relative position of rickettsiae in the cytoplasm above the nucleus is clearly evident (Fig. 1c, right, small triangles). Incorporation of symbionts into pole cells has been previously described for the mosquito, *Aedes polynesiensis*, indicating a similar mechanism of vertical transmission¹⁸. During the syncytial blastoderm stages, nuclei are surrounded by rickettsiae in the regions known to be organized by microtubule cytoplasmic domains¹⁹. During mitosis (Fig. 1d), rickettsiae appear to align with the spindle axis (double headed arrow in Fig. 1d), possibly by specific interactions with the cytoskeleton.

Rickettsiae are not detectable in the egg following treatment of fly stocks with tetracycline. This observation correlates with the loss of CI in these treated strains as shown in Table 1. If both males and females are treated with tetracycline, no rickettsiae are observed in their eggs, and normal development proceeds (Fig. 2a). CI is, however, still observed in crosses where the female has been treated, but not the male (Fig. 2b). Figure 2b shows aberrant nuclear divisions (arrows) with no evidence of rickettsiae in the egg.

Fertilized eggs of reciprocal incompatible crosses between strains isolated from California and Hawaii (Fig. 2c and d) show division defects indistinguishable from those seen in Fig. 2b. Both images show the apparent disintegration of the few cleavage nuclei present at this stage of development. We also observe that the rickettsiae appear to coalesce around the fragmenting DNA (arrowhead in Fig. 2d). Therefore, in all cases of CI we observe early developmental defects in the cleavage products. These observations are in agreement with previous work on mosquitoes of the *Culex pipiens* complex²⁰.

Figure 3 shows examples of newly fertilized eggs derived from crosses between either tetracycline-treated males and females (Fig. 3a), or untreated males crossed to tetracycline-treated

FIG. 1 DNA-positive bodies of *Rickettsia*-infected fertilized eggs derived from a stock of *Drosophila simulans* exhibiting cytoplasmic incompatibility. *a*, Surface view of a whole mount embryo fixed at the nuclear cleavage stages showing a large concentration of small punctate DNA-positive bodies localized to the cortex; *b*, punctate bodies surrounding cleavage nuclei (arrow, larger brightly staining structures) in the interior of a similarly staged embryo; *c*, punctate bodies, presumably rickettsiae, segregating into the pole buds immediately before pole cell formation. The left panel of *c* is a Nomarski image of pole buds (n, nucleus), the right panel shows the identical field of view under fluorescent optics, small triangles indicate the position of the rickettsiae located in the cytoplasm above the nucleus; *d*, embryo at the syncytial blastoderm stage fixed during mitosis. DNA-positive bodies are asymmetrically localized around the chromosomes and are aligned with the axis of the mitotic spindle. Spindle axis indicated by the double-headed arrow.

METHODS. *D. simulans* (from a strain originally collected near Riverside and known to display cytoplasmic incompatibility⁸) were reared in population cages. Collections of fertilized eggs were fixed, stained and mounted accord-

ing to established protocols¹⁹. DNA was stained using the DNA-specific dye, DAPI, and observed with a Zeiss Axioplan microscope equipped with epifluorescence optics and recorded on 35 mm Technical Pan 2415 film (Kodak). All eggs were shown to be fertilized by immunofluorescent staining of the sperm tail using a sperm-specific monoclonal antibody (T.L.K., manuscript submitted for publication).

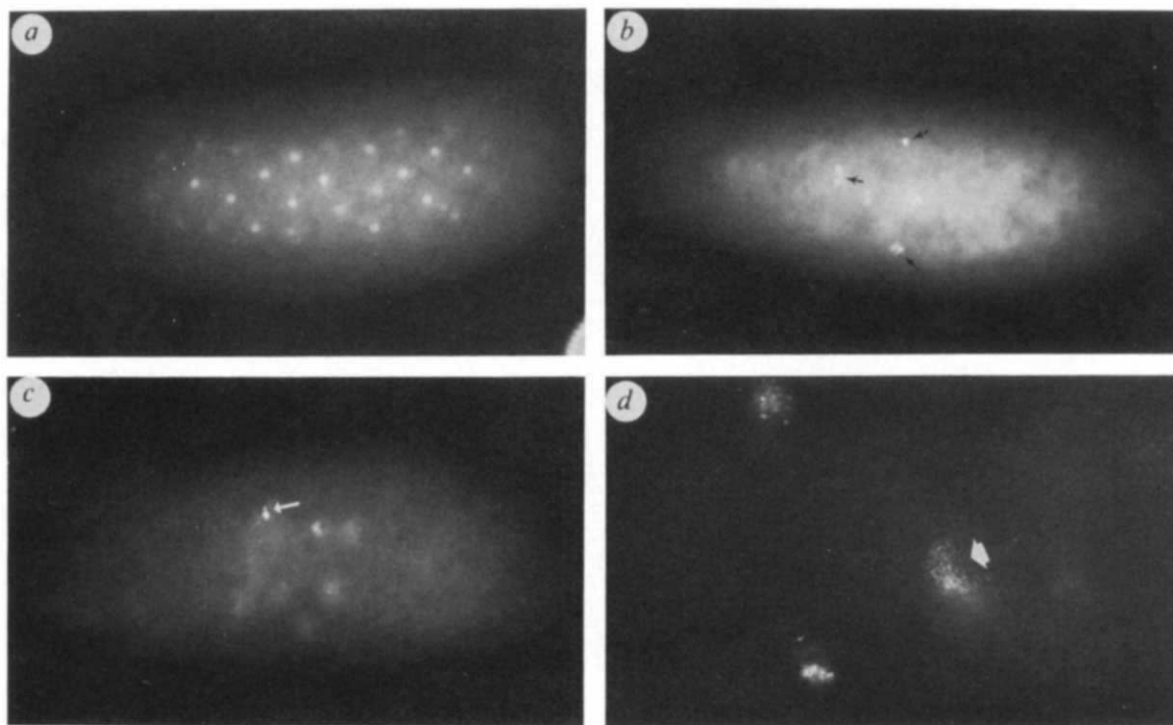


FIG. 2 Eggs displaying *a*, normal development; *b*, unidirectional incompatibility and *c*, *d*, bidirectional incompatibility in *D. simulans*. *a*, Normal development in an embryo derived from parents previously treated with tetracycline; *b*, embryo derived from a cross between a tetracycline-treated female and an untreated male (arrows point to defective cleavage products); *c* and *d*, early

developmental defects in eggs derived from bidirectionally incompatible crosses between two different strains of *D. simulans* (DSR and DSH in Table 1). Fixation, staining and observation were as described in the legend to Fig. 1. Tetracycline was administered as described in Table 1.

TABLE 1 Adult progeny from *D. simulans* crosses between individuals from Hawaii and California and tetracycline-treated derivatives from these strains

Cross ♀ × ♂	Unidirectional intrastrain incompatibility (California)			Cross ♀ × ♂	Unidirectional intrastrain incompatibility (Hawaii)			Bidirectional interstrain incompatibility			
	$\bar{x}$	s.e.m.	n		$\bar{x}$	s.e.m.	n	Cross ♀ × ♂	$\bar{x}$	s.e.m.	n
DSR × DSR	58.0	2.7	(18)	DSH × DSH	57.4	7.9	(9)	DSH × DSR	1.6	0.7	(17)
DSR × DSRT	55.9	2.3	(17)		33.7	7.7	(6)	DSHT × DSRT	47.7	7.6	(6)
DSRT × DSRT	63.1	5.5	(14)		53.5	4.1	(11)	DSR × DSH	6.0	3.7	(9)
DSRT × DSR	4.6	1.7	(12)		8.9	2.8	(12)	DSRT × DSHT	53.7	4.3	(12)

Flies were maintained at laboratory temperature on standard cornmeal-molasses diet media except when tetracycline-supplemented media were required. In this case standard medium was melted and 4% tetracycline hydrochloride was added to a final concentration of 0.025%. After one generation on tetracycline-supplemented media flies were returned to standard media for at least one generation before being used in any experiments. Crosses were established between one female and two to three males and left for 6 days before the vials were cleared. Vials in which fewer than 10 eggs had been laid were discarded. Adult progeny were then scored on emergence. DSR: *D. simulans* Riverside; DSRT: *D. simulans* Riverside, tetracycline-treated; DSH: *D. simulans* Hawaii; DSHT: *D. simulans* Hawaii, tetracycline-treated. s.e.m., Standard error of the mean; n, number of vials counted.

* $P < 0.001$; NS, $P > 0.001$ Mann-Whitney.

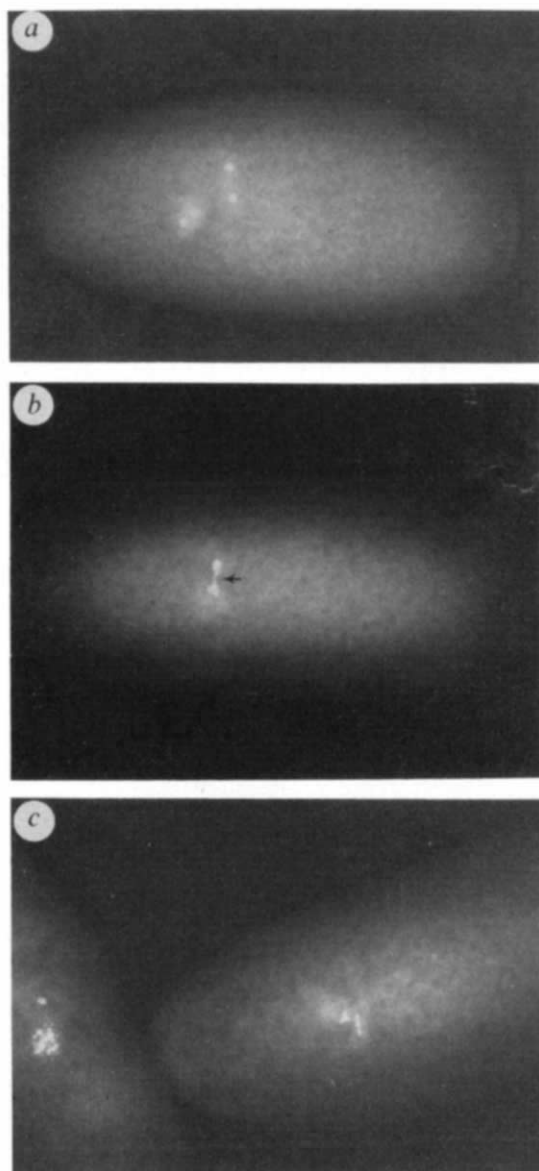


FIG. 3 Early embryological defects associated with CI. Eggs fertilized by sperm from *a*, tetracycline-treated parents (normal development), or *b*, *c*, untreated males. The arrow in *b* points to an aberrant cytoplasmic bridge formed during the first cleavage division (gonomeric division). Parts of two embryos in *c* show, (left) highly fragmented DNA, and (right) two condensed DNA bodies that are probably the result of failed karyogamy. Methods as for Fig. 1.

females (Fig. 3*b* and *c*). All three images are devoid of rickettsiae, as judged by the lack of any observable DAPI-positive bodies (compare Fig. 1*a*). Normal development in Fig. 3*a* is judged by the normal nuclear morphology observed and by comparison with early developmental stages observed in *D. melanogaster*²¹. Aberrant DNA structures are clearly visible in a high percentage (>90%) of incompatible eggs (Fig. 3*b* and *c*). Of particular note is the presence of an abnormal 'mitotic bridge' during the first cleavage division (Fig. 3*b*) and the presence of two smaller, condensed DNA bodies in Fig. 3*c* (the degraded DNA in the egg on the left in Fig. 3*c* is presumably the breakdown product of an earlier abortive cleavage division). The mitotic bridge bears a striking resemblance to defects observed in a paternal-effect lethal mutation of *D. melanogaster*, ms(3)K81²² (T.K., unpublished data). In addition, the pleiotropic phenotypes observed in Fig. 2*b*, *c* and *d*) are also shown in ms(3)K81-affected eggs, suggesting that they may both be affecting similar cellular processes.

The phenotype of both unidirectional and bidirectional CI in *D. simulans* appears identical to CI in *Culex pipiens*. The existence of bidirectional incompatibility in *Drosophila*, however, provides an accessible genetically well defined system to investigate the molecular and cellular mechanisms involved in this phenomenon. That CI is associated with sperm-derived functions emphasizes the importance of paternal contributions to sperm-egg interactions. CI in *Drosophila* may provide a model system for understanding a potentially novel means of pest control as well as the processes involved in the early development of insects.

Note added in proof: We note that similar microorganisms have been observed in *D. melanogaster* (see Scientific Correspondence, this issue²³).

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- Laven, H. *Cold Spring Harb. Symp. quant. Biol.* **24**, 166-173 (1959).
- Wade, M. J. & Stevens, L. *Science* **227**, 527-528 (1985).
- O'Neill, S. L. *J. Invertebr. Path.* **53**, 132-134 (1989).
- Hsiao, C. & Hsiao, T. H. *J. Invertebr. Path.* **45**, 244-246 (1985).
- Kellen, W. R., Hoffman, D. F. & Kwock, R. A. *J. Invertebr. Path.* **37**, 273-283 (1981).
- Richardson, P. M., Holmes, W. P. & Saul, G. B. *J. Invertebr. Path.* **50**, 176-183 (1987).
- Noda, H. *Entomologia exp. appl.* **35**, 263-267 (1984).
- Hoffmann, A. A., Turelli, M. & Simmons, G. M. *Evolution* **40**, 692-701 (1986).
- Hoffmann, A. A. *Entomologia exp. appl.* **48**, 61-67 (1988).
- Yen, J. H. & Barr, A. R. *J. Invertebr. Path.* **22**, 242-250 (1973).
- Breeuwer, J. A. J. & Werren, J. H. *Nature* **346**, 558-560 (1990).
- Curtis, C. F. *et al. Entomologia exp. appl.* **31**, 181-190 (1982).
- Laven, H. *Nature* **216**, 383-384 (1967).
- Rao, T. R. *J. Commun. Dis.* **6**, 57-72 (1974).
- Curtis, C. F. *Bull. Wild Hlth Org.* **53**, 107-119 (1976).
- Binnington, K. C. & Hoffmann, A. A. *J. Invertebr. Path.* **54**, 344-352 (1989).
- Zalokar, M. & Erk, I. *J. Microbiol.* **25**, 97-106 (1976).
- Wright, J. D. & Barr, A. R. *J. Invertebr. Path.* **38**, 409-418 (1981).
- Karr, T. L. & Alberts, B. M. *J. Cell Biol.* **102**, 1494-1509 (1986).
- Jost, E. *Can. J. Genet. Cytol.* **13**, 237-250 (1971).
- Karr, T. L., Weir, M. P., Ali, Z. & Kornberg, T. *Development* **105**, 605-612 (1989).
- Fuyama, Y. *Genetics* **112**, 237-248 (1986).
- Glover, D. M. *et al. Nature* **348**, 117 (1990).

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